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Effects of nitrification inhibitors and application technique on trace gas fluxes from a maize field after cattle slurry fertilization

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Dissertation

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Frequently used abbreviations

°C	degree Celsius
AMO	ammonia monooxygenase
ANAMMOX	anaerobic ammonium oxidation
C	carbon
CAN	calcium ammonium nitrate
$CaCl_2$	calcium chloride
CH_3COOH	acetatic acid
CH_4	methane
Cl	chloride
CO_2	carbon dioxide
CON	unfertilized control
Cu	copper
DCD	dicyandiamide
DM	dry matter
DMP	dimethylpyrazole
DMPP	3,4-dimethylpyrazole phosphate
DMPSA	3,4-dimethylpyrazole succinic acid
DNRA	dissimilatory nitrate reduction to ammonium
ECD	electron capture detector
EF	emission factor
Fe^{3+}	ferric
FID	flame ionization detector
FM	fresh matter
GHG	greenhouse gas
GSW	gravimetric soil water content
GWP	global warming potential
H_2	atomic hydrogen

HAO	hydroxylamine oxidoreductase
HFC	hydrofluorocarbons
INC	slurry incorporation
INJ	slurry injection
K_2SO_4	potassium sulfate
M	molar
MFE	mineral-fertilizer N equivalents
MIN	mineral fertilization with CAN
MMO	methane monooxygenase
mV	millivolt
N	nitrogen
n	sampling size
N_2	molecular nitrogen
N_2O	nitrous oxide
NH_2OH	hydroxylamine
NH_3	ammonia
NH_4^+	ammonium
NI(s)	nitrification inhibitor(s)
Nitrapyrin	2-Chloro-6-(trichloromethyl)-pyridine
N_{min}	mineral N
NO	nitrogen monoxide
NO_2^-	nitrite
NO_3^-	nitrate
nosZ	gene sequence encoding for N_2O reductase
NUE	nitrogen use efficiency
O_2	oxygen
O_3	ozone
OH	hydroxyl

OM	organic matter
PFC	perfluorocarbon
ppb	part per billion
ppm	part per million
r^2	correlation coefficient
SE	standard errors
SF_6	sulfur hexafluoride
SO_4^{2-}	sulfate
SOM	soil organic matter
TAN	ammoniacal nitrogen
TZ & MP	1,2,4 Triazole and 3-Methylpyrazole
WFPS	water filled pore space

1 Summary – Zusammenfassung

1.1 Summary

In a time of climate change and against the background of intensive animal husbandry and biogas production in Germany, strategies for mitigation of greenhouse gas (GHG) release and Nitrogen (N) losses from silage maize production become increasingly important, especially for organic fertilizers. Consequently, the **main objective** of this study was to determine the height of GHG release from silage maize production on a medium textured soil which is typical for this region in Southwest Germany and to evaluate useful fertilization opportunities to mitigate carbon dioxide (CO_2) footprint per yield unit.

GHG emissions from arable soils worldwide, such as nitrous oxide (N_2O) or methane (CH_4) contribute to atmospheric composition. N-fertilizers stimulate N_2O formation in soils by providing substrate for microbial processes, such as nitrification and denitrification. Ammonia (NH_3) volatilizations and nitrate (NO_3^-) leaching are also enhanced through N-fertilization and contribute to N_2O emissions indirectly by being re-emitted as N_2O. Concerning CH_4 budget from soils, aeration is the key factor determining whether soils are CH_4 sources or sinks. Generally, in well-aerated arable soils, CH_4 oxidation is the dominant microbial process resulting in net CH_4 uptakes. Conversely, under predominant anaerobic conditions favoring CH_4 formation by methanogens, net CH_4 emissions are recorded. To identify management factors improving GHG budget from silage maize, annual N_2O and CH_4 measurements were carried out during maize growth and subsequent black fallow at least weekly with the closed chamber method. Investigations were conducted over two years on two adjacent fields (one for each study year). Amounts of NH_3 volatilizations after fertilization and NO_3^- leaching losses were also included in GHG balances. In dependence on available data, determined or estimated values were used. Additionally, yield and N removal from maize plants were quantified.

The basic treatments of this study which investigated impact of fertilizer form and application techniques, were an unfertilized control (CON), a mineral fertilization (MIN), a banded cattle slurry application by trailing hose and subsequent incorporation (INC) and a cattle slurry injection (INJ). As confirmed repeatedly, in contrast to broadcast slurry incorporation, slurry injection efficiently reduced the risk of NH_3 losses by direct slurry placement into the soil, but simultaneously provoked N_2O formation more strongly, probably due to the anaerobic conditions in the injection slot favoring denitrification. For reducing N_2O release from slurry injection, the applicability of six single or combined nitrification inhibitors (NIs) concerning potential GHG reduction were investigated. This N_2O reduction should be reached through the desynchronized availability of carbon (C) and NO_3^-, derived from nitrified slurry ammonium (NH_4^+). Thus, in

the period after slurry application, N_2O losses from denitrification as well as from nitrification should be reduced through NIs.

For final evaluation, collection of measured and estimated data (including direct and indirect N_2O losses (NH_3, NO_3^-), CH_4 budget, pre-chain emissions from mineral fertilizer and fuel consumption) were converted into CO_2 equivalents and summarized as area- or yield-related GHG balances. Except for one of the INJ treatments with NI (exclusively investigated in the first year) and one INC treatment with NI (exclusively investigated in the second year), all remaining treatments were tested in both experimental years.

The height of **NH_3** emissions from INC treatment (12-23 % of applied NH_4^+-N) was more weather-dependent than those from INJ treatment (12-15 % of applied NH_4^+-N). In mean over both years, cumulative **N_2O** emission from INJ treatment (13.8 kg N_2O-N ha^{-1} yr^{-1}), was significantly higher than from CON, MIN, and INC which recorded 2.8, 4.7, and 4.4 kg N_2O-N ha^{-1} yr^{-1}. NIs decreased the fertilization-induced N_2O emissions from injection by 36 % (mean over all NIs and years) by an order of magnitude comparable to slurry incorporation. However, differing NIs tended to vary in their N_2O reduction potentials, probably due to different chemical and physical characteristics. The NIs investigated tended to be categorized in inhibitors with prior and delayed inhibitory maximum. Whether low persistence, or poor biological degradability was an advantage, depended on environmental conditions. A combination of two NIs, one with putative prior and one with delayed release behavior reached the highest N_2O reduction, probably by covering the longest time period and lowering the impact of unpredictable environmental factors. In the additional INC treatment, this NI combination tended to reduce annual N_2O release by 20 % in comparison to incorporation without inhibitor.

Beside the potential of reducing fertilization-induced N_2O emissions, NIs might also help to improve **CH_4** budgets in silage maize production. In general, CON, MIN and INC were net CH_4 sinks in both years with mean uptakes of 460, 127, and 793 g CH_4-C ha^{-1} yr^{-1}, respectively. Conversely, slurry injection resulted in net CH_4 emissions of 3144 g CH_4-C ha^{-1} yr^{-1} (mean over both years). However, NIs tended to reduce CH_4 emissions from injection by around 48 % and increased CH_4 consumption from slurry incorporation by 20 %.

Due to the amount of inorganic N applied in the MIN treatment was twice as much as in the organically fertilized treatments, exclusively mineral fertilized maize plants showed significantly higher **N removal**.

Slurry-fertilized treatments were neither affected by application technique, nor by any of the NIs, probably due to the high N doses masking the beneficial effects from NIs.

However, as shown through an experiment with sunflowers, one year after treatment-specific fertilization, in comparison to sole slurry injection, the residual effect from INJ treatments with NIs was partially increased.

Across all treatments and years, direct N_2O emissions were the major contributor to total **GHG balance**. Yield-related GHG budgets from both years were lowest for CON, followed by INC or MIN treatment and significantly highest for sole slurry injection. NIs decreased fertilization-induced GHG release from injection in mean over both years by order of magnitude comparable with slurry incorporation.

Consequently, alongside slurry incorporation and broadcast mineral fertilization, slurry injection combined with recommended NIs was evaluated as an equally appropriate fertilization strategy in terms of the atmospheric burden for livestock farmers.

1.2 Zusammenfassung

In Zeiten des Klimawandels und vor dem Hintergrund einer intensiven Tierhaltung und Biogasproduktion in Deutschland gewinnen Treibhausgas- (THG) Emissionen und Stickstoff (N)-Verluste in der Silomaisproduktion, insbesondere beim Einsatz organischer Düngemittel zunehmend an Bedeutung. **Ziel dieser Studie** war es daher, den THG-Ausstoß für die Silomaisproduktion in einer Region Südwestdeutschlands auf einem für diese Region typischen mittelschweren Boden zu quantifizieren und anhand unterschiedlicher Düngungsstrategien klimafreundliche Methoden mit möglichst geringem CO_2-Fußabdruck herauszustellen.

Insbesondere Lachgas- (N_2O) und Methan- (CH_4) Emissionen tragen elementar zum globalen THG-Ausstoß von Ackerböden und somit zum Klimawandel bei. Die N_2O-Bildung in Böden wird vor allem durch den Einsatz von N-Düngern gefördert, da diese das Substrat für mikrobielle N-Umwandlungsprozesse wie Nitrifikation und Denitrifikation bereitstellen. Aber auch Ammoniak- (NH_3) Emission und Nitrat- (NO_3^-) Auswaschung werden durch die N-Düngung beeinflusst und tragen zu einer weiteren Erhöhung des N_2O-Ausstoßes indirekt bei. Diese indirekten N_2O-Emissionen entstehen, da ein Teil der N-haltigen Nährstoffeinträge in umliegende Ökosysteme, dort ebenfalls zu einer zusätzlichen N_2O-Bildung führt. Ob ein Boden eine CH_4- Quellen oder –Senken-Funktion hat, entscheidet meist der Belüftungszustand des Bodens. Im Allgemeinen sind gut belüftete Ackerböden eine CH_4- Senke, da die CH_4- Oxidation ungestört ablaufen kann. Dominieren hingegen anaerobe Bodenbedingungen, läuft vermehrt der Prozess der Methanogenese ab und es kommt zu Netto-CH_4-Emissionen.

Zur Quantifizierung der THG-Bilanzen von Silomais mit unterschiedlichen Reduktionsansätzen, wurden durchgehend über zwei Jahre hinweg mindestens einmal wöchentlich N_2O- und CH_4- Flüsse mit der geschlossenen Kammermethode gemessen. Zudem wurden indirekte N_2O Emissionen (NH_3, NO_3^-) erfasst oder geschätzt sowie Silomaiserträge und N-Abfuhren ermittelt. Für jedes der beiden Versuchsjahre wurde hierfür ein separates Versuchsfeld mit folgenden Basis-Behandlungen angelegt: ungedüngte Kontrolle (CON), mineralische Düngung (MIN), Rindergüllenapplikation mittels Schleppschlauch und anschließender Einarbeitung (INC) und Rindergülleninjektion (INJ). Im Gegensatz zur oberflächigen Gülleapplikation mit breitflächiger Einarbeitung ist die Gülleinjektion dafür bekannt, NH_3-Verluste durch die direkte Platzierung in den Boden effektiver zu mindern, im Gegenzug allerdings die N_2O-Bildung aufgrund der meist stark anaeroben Bedingungen im Injektionsschlitz stärker zu fördern. Um die vermehrte N_2O-Produktion dieser Applikationstechnik zu vermindern, wurde der Einsatz sechs verschiedener Nitrifikationsinhibitoren (NIs) zur strategischen THG-Minderung zusätzlich untersucht. Dies sollte durch die zeitliche Entzerrung von Kohlenstoff- (C) und NO_3^--Angebot nach Gülleapplikation aufgrund einer verlängerten Stabilisierung des

Ammoniums (NH_4^+) aus der Gülle erzielt werden und somit die N_2O-Verluste aus der Denitrifikation als auch aus der Nitrifikation reduzieren. Abgesehen von einer INJ-Variante mit NI, die im zweiten Jahr durch eine zusätzliche INC-Variante mit NI ersetzt wurde, wurden alle übrigen Behandlungen in beiden Jahren untersucht.

Für die abschließende Bewertung der getesteten Düngungsstrategien wurden die zur THG-Bilanz beitragenden Posten in CO_2-Äquivalente umgewandelt und konnten schließlich auf Flächen- oder Ertragseinheiten bezogen werden. Hierbei wurden direkte und indirekte N_2O-Emissionen (NH_3, NO_3^-), CH_4-Budget, Vorketten-Emissionen für die Mineraldüngerbereitstellung und der Dieselverbrauch für Feldmaßnahmen berücksichtigt.

Die Höhe der **NH_3**-Emissionen der INC-Behandlung (12-23 % des gedüngten NH_4^+-N) war stärker von den Witterungsverhältnissen abhängig als die der INJ-Behandlung (12-15 % des gedüngten NH_4^+-N). Kumulative **N_2O**-Emissionen der Gülleinjektion betrugen im Mittel über beide Jahre 13.8 kg N_2O-N ha^{-1} $Jahr^{-1}$ und lagen damit signifikant über dem Mittel der CON-, MIN-, oder INC-Behandlung, die Emissionen in Höhe von 2.8, 4.7 und 4.4 kg N_2O-N ha^{-1} $Jahr^{-1}$ verzeichneten. Der Einsatz von NIs reduzierte die jährlichen N_2O-Emissionen der Gülleinjektion im Durchschnitt über alle NIs und Jahre um 36 % und machte die Gülleinjektion mit der INC-Behandlung somit vergleichbar. Allerdings schwankten die einzelnen NIs hinsichtlich ihres N_2O-Reduktionspotentials, vermutlich aufgrund unterschiedlicher chemischer und physikalischer Eigenschaften. Innerhalb dieser Studie konnten die verwendeten NIs tendenziell in zwei Gruppen eingeteilt werden. Eine Gruppe mit vermeintlich geringerer Persistenz und früherem Inhibierungsmaximum im Boden und eine Gruppe, deren Substanzen ihre volle Wirkung erst zu einem späteren Zeitpunkt entfalteten. Welche Gruppe eine vermeintlich höhere Inhibierungswirkung aufwies, hing stark von den Umweltbedingungen des jeweiligen Versuchsjahres ab. Die INJ-Variante, die sowohl ein NI mit früherem und späterem Inhibierungsmaximum enthielt, verzeichnete tendenziell das höchste N_2O-Reduktionspotential, wahrscheinlich aufgrund der Dauer des Inhibierungszeitraums und der geringeren Beeinflussbarkeit durch Umweltfaktoren. Für die zusätzliche INC-Behandlung mit NI, wurde ebenfalls diese Wirkstoffkombination angewendet, sie erzielte eine N_2O-Reduktion von 20 % im Vergleich zur Gülleeinarbeitung ohne Hemmstoff.

Neben dem Potential düngungs-induzierte N_2O-Emissionen zu reduzieren, stellte sich der Einsatz von NIs auch für das **CH_4**-Budget als vorteilhaft heraus. Die Behandlungen CON, MIN und INC zeigten in beiden Jahren eine Netto-CH_4-Aufnahme mit durchschnittlich 460, 127 und 793 g CH_4-C ha^{-1} $Jahr^{-1}$. Im Gegensatz dazu führte eine Gülleinjektion in beiden Jahren zu Netto-CH_4-Emissionen mit durchschnittlich 3144 g CH_4-C ha^{-1} $Jahr^{-1}$. Der Einsatz von NIs reduzierte die Höhe dieser CH_4-Emissionen allerdings

um rund die Hälfte. Außerdem führte eine inhibierte Nitrifikation bei breitflächiger Gülleeinarbeitung tendenziell zu einer Erhöhung der CH_4-Aufnahme um 20 %.

Da die Menge an mineralisch gedüngtem N in der MIN-Variante doppelt so hoch war wie in den organisch gedüngten Varianten (INC, INJ), verwundert es nicht, dass dadurch eine signifikant höhere **N-Abfuhr** mit dem Erntegut vom Feld erzielt wurde. Organisch gedüngte Behandlungen waren weder von der Applikationstechnik, noch durch den Gebrauch von NIs hinsichtlich ihrer N-Abfuhr signifikant beeinflusst. Allerdings wurde bei einem zusätzlichen Versuch mit Sonnenblumen, der ein Jahr nach behandlungs-spezifischer Düngung auf der Versuchsfläche des ersten Jahres angelegt wurde, eine erhöhte N-Abfuhr erfasst, wenn die vorherige Gülleinjektion mit NI-Zugabe erfolgt war.

Die direkten N_2O-Emissionen hatten, unabhängig von Behandlung oder Jahr, den größten Anteil an der **THG-Bilanz**. Die geringste ertragsbezogene THG-Bilanz ergab sich für die ungedüngte Kontrollvariante, gefolgt von breitflächiger organischer oder mineralischer Düngung, die höchste ergab sich für die Gülleinjektion. Allerdings konnten die rein auf die Düngungsmaßnahme bezogenen THG-Emissionen der Gülleinjektion durch den Einsatz von NIs im Mittel über beide Jahre auf ein THG-Niveau abgesenkt werden, das mit der breitflächigen Gülleeinarbeitung vergleichbar war.

Somit stellte sich neben einer mineralischen oder oberflächigen Gülleapplikation mit möglichst zeitnaher Einarbeitung, auch die Gülleinjektion als vergleichsweise THG-freundliche Applikationstechnik heraus, vorausgesetzt einer der empfohlenen NIs wurde vorab zugegeben.

2 General Introduction – state of research

2.1 Climate change

The concentration of atmospheric trace gases such as carbon dioxide (CO_2), nitrous oxide (N_2O), methane (CH_4), ozone (O_3) and others (*e.g.* water vapor the most important natural greenhouse gas (GHG)) plays one of the key roles influencing global climate (*Myhre et al.*, 2013). Through their properties of allowing short-wave solar radiation to fall onto earth almost unhindered and in turn to absorb long-wave radiation emitted from the warm earth, they exhibit an energetic state for a certain time period emitting infrared radiation of which a substantial share has a heating effect and thus results in global warming. This natural GHG effect is vital for the existence of life on planet earth by increasing mean global air temperature from around -18 °C to 15 °C (*UBA*, 2017).

However, since the beginning of industrialization, human activity leads to strong increases (Figure 2.1) and changes in the composition of atmospheric GHGs.

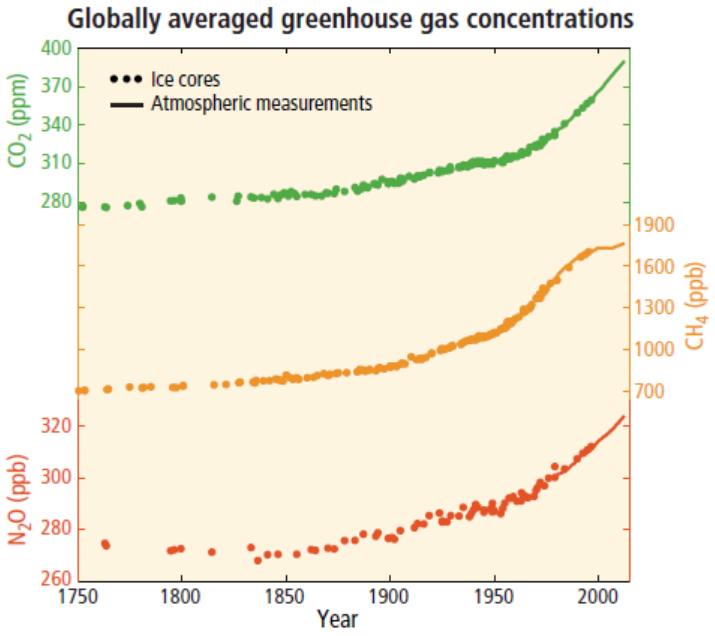

Figure 2.1: Observed changes in atmospheric greenhouse gas concentrations. Atmospheric concentrations of carbon dioxide (CO_2, green), methane (CH_4, orange), and nitrous oxide (N_2O, red). Data from ice cores (symbols) and direct atmospheric measurements (lines) are overlaid (*IPCC*, 2014a).

Thus, the globally averaged atmospheric CO_2, N_2O and CH_4 concentrations increased in the period from 1750 to 2015 by 43 %, 20 %, and 150 %, respectively. Moreover, new molecules contributing to greenhouse effect, such as hydrofluorocarbons (HFCs), perfluorocarbons (PFCs) and sulfur hexafluoride (SF_6) were released into the atmosphere during the last decades (*Blasing*, 2014).

This anthropogenic induced rise of atmospheric GHG concentration reinforced the natural GHG effect and is very likely to be the primary factor for global warming which has raised the lower atmosphere's temperature by 1 °C since 1750 (*UBA*, 2017). Furthermore, the continuing rise of the oceans' temperature, thawing of glaciers and permafrost soils, melting icecaps and increasing sea levels are further consequences of climate change. To prevent more dangerous effects of climate change with ecological and societal impacts, global warming is limited to an increase of less than 2 °C when compared to pre-industrial level (*Tanaka et al.*, 2013).

For non-exceeding critical concentration of atmospheric GHGs, the United Nations Framework Convention on Climate Change regulated GHG release for the first time through the Kyoto Protocol which requires the reduction of certain GHGs within certain time frames for industrialized countries (*UBA*, 2017). Thus, the reduction of GHGs was declared a national and an international political objective to protect the earth's climate, human civilization and the environment (*UBA*, 2017).

In 2012, Germany reached, and considerably exceeded the expectation of the provisional target for that year by reducing the GHG by 24.3 % instead of the required target of 21 % with respect to the pre-industrial emission level (*Shishlov et al.*, 2016). However, the final objective of reducing GHG emissions by 50% from the basic year 2000 until 2050 will not easily be achieved within the following decades. Thus further strategies reducing GHG release from varying source categories and sectors are required (*UBA*, 2017). As the spatial variation of GHG source groups vary dependent on country or region, likewise the mitigation strategies of the site-specific GHG release differ.

In Germany, CO_2 has the highest share on the GHG inventory with 87.8 %, mainly attributed to the mobile and stationary combustion of fossil fuels. CH_4 release has a share of 6.2 % on the German GHG inventory which is primary attributed to animal husbandry. N_2O emissions accounting for 4.3 % and have their highest reduction potential in their main source, the agricultural sector, in particular in reducing N_2O release from agricultural soils (*UBA*, 2017).

In this wide field of GHG mitigation, this PhD study is focused on the potential of reducing GHG emissions from silage maize production on a medium textured soil in Southwest Germany by varying management factors, such as fertilizer form, application technique and/or the addition of additives inhibiting nitrification. Therefore, N_2O and CH_4 fluxes were measured over two entire years in a field study. Indirect N_2O emissions derived from NH_3 emissions were measured and other indirect GHG emissions, such as fuel consumption and nitrate (NO_3^-) leaching-induced GHG releases were calculated using standard default values. For evaluating the varying management factors regarding yield-related GHG balance and internal nitrogen (N) cycle, maize yield and N removal

were likewise determined. To contribute to an understanding of how the measured variables might be influenced by environmental and management factors, firstly, the microbial production and consumption processes of N_2O and CH_4 are described briefly below. In addition, the formation of indirect N_2O emissions induced by ammonia (NH_3) emission will be clarified. Lastly, NIs as a strategy for intervening in GHG release and N cycle will be introduced in this chapter depending on the characteristics of the active compounds and environmental conditions.

2.2 Nitrous oxide

2.2.1 Contribution to climate change

The trace gas N_2O contributes substantially to climate change with a share of 7.9 % of total annual GHG emissions attributed to human perturbations (*Rogner et al.*, 2007). Despite its low atmospheric concentration, the high contribution of N_2O to climate change is caused by its absorption ability of infrared radiation and its high stability in the troposphere (residence time of 114 years) (*UBA*, 2017). As a result of its atmospheric lifetime, N_2O is able to diffuse and to accumulate in the stratosphere, where N_2O reacts with photochemically produced oxygen radicals ($O^{\cdot}$) to nitrogen monoxide (NO), which leads to ozone depletion by building nitrite (NO_2^-) and O_2 (*Crutzen*, 1970). *Ravishankara et al.* (2009) predict N_2O as the dominant trace gas for stratospheric ozone destruction in the 21th century.

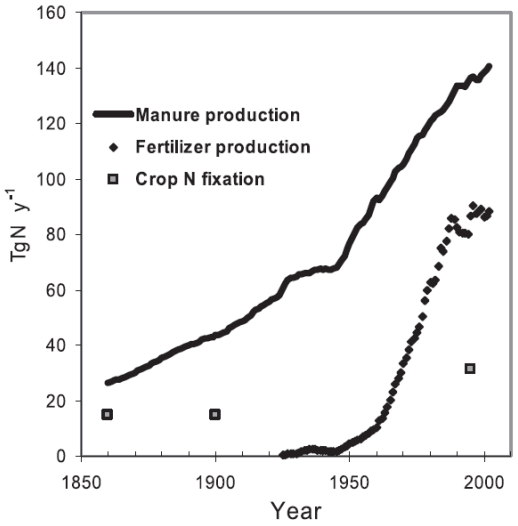

Figure 2.2: Changes in the indices of the global agricultural N cycle since 1850: the production of manure, fertilizer and estimates of crop N fixation (*Denman et al.*, 2007).

The strong increase of atmospheric N_2O concentration from the pre-industrial level of 270 ppb to 324 ppb in 2011 (*Myhre et al.*, 2013) can mainly be attributed to human activity. Thereby, especially the increased N input in N cycle through fixation of atmospheric molecular nitrogen (N_2) by the Haber-Bosch process pushed the level of global

N fixation by almost one third per year (Figure 2.2) (*Fowler et al.*, 2013). This synthetically produced N is mainly used as N-fertilizer in agriculture. However, only a small proportion of the N fertilized leaves the field bounded in harvested crops (*Cassman et al.*, 2002).

This leakage of N not used for biomass sequestration results in agriculture as the major source of global anthropogenic N_2O emissions (*Bouwman et al.*, 2002; *Hu et al.*, 2015; *Smith et al.*, 2008) (Table 2.1).

Also in Germany, the agricultural sector was confirmed as the single biggest N_2O source with a share of 81 % on total national anthropogenic N_2O emission in 2015 (*UBA*, 2017). With 22 % and 12 %, especially the application of mineral N and manure contributed substantially to the total national anthropogenic N_2O emissions (*UBA*, 2017).

The high share of N_2O produced in cultivated soils is mainly attributed to microbial conversion processes forming N_2O, like nitrification, denitrification or nitrifier-denitrification (*Arp and Stein*, 2003; *Azam et al.*, 2002; *Wrage et al.*, 2001) (Figure 2.3). The microbial pathways of N_2O production and/or consumption are described briefly in the following section.

Table 2.1: Global sources of N_2O for the 1990s (*Denman et al.*, 2007).

Source	**N_2O [Tg N yr^{-1}]**	**range**
Anthropogenic sources		
Fossil fuel combustion & industrial processes	0.7	0.2 - 1.8
Agriculture	2.8	1.7 - 4.8
Biomass and biofuel burning	0.7	0.2 - 1.0
Human excreta	0.2	0.1 - 0.3
Rivers, estuaries, coastal zones	1.7	0.5 - 2.9
Atmospheric deposition	0.6	0.3 - 0.9
Anthropogenic total	**6.7**	
Natural sources		
Soils and natural vegetation	6.6	3.3 - 9.0
Oceans	3.8	1.8 - 5.8
Atmospheric chemistry	0.6	0.3 - 1.2
Natural total	**11.0**	
Total sources	**17.7**	**8.5 - 27.7**

2.2.2 Processes of N_2O formation in soils

As shown in Figure 2.3, numerous biotic processes are responsible for N_2O formation and consumption in soils including heterotrophic denitrification, nitrification, nitrifier denitrification, dissimilatory nitrate reduction to ammonium (DNRA) or the anaerobic ammonium oxidation (ANAMMOX) (*Butterbach-Bahl et al.*, 2013). Despite these var-

ious biotic processes forming N_2O in ecosystems, in literature, nitrifying pathways (including nitrification and nitrifier-denitrification) and denitrification are predominantly characterized as the two main sources of N_2O production in agricultural soils (*Hu et al.*, 2015). In soil compartments with low O_2 partial pressure, denitrification is in principal the dominant process for N_2O release. Under aerobic soil conditions, nitrification-related pathways are generally the major contributors to N_2O emissions, whereby the total amount emitted is usually lower than under conditions favoring denitrification (*Bateman and Baggs*, 2005; *Firestone and Davidson*, 1989). The major part of the global N_2O release in agricultural soils is attributed to denitrification (*Giles et al.*, 2012).

In this study, the focus is on these two main processes of biotic N_2O formation. Processes of abiotic N_2O production, such as chemodenitrification, chemical decomposition of hydroxylamine (NH_2OH) or surface decomposition of ammonium nitrate (NH_4NO_3) (*Butterbach-Bahl et al.*, 2013) are not discussed further.

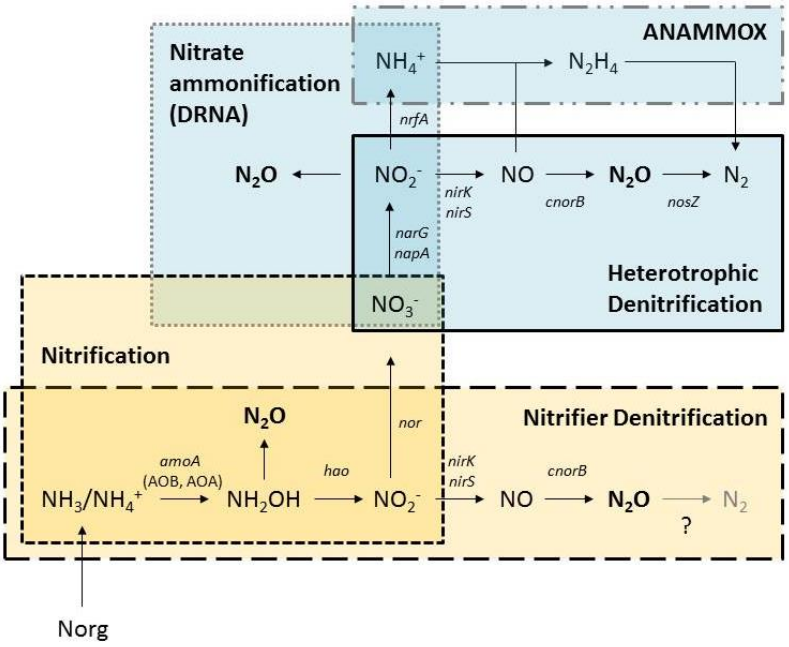

Figure 2.3: N cycling processes involved in N_2O production and reduction (*Krauß*, 2018).

2.2.2.1 Denitrification

Denitrification is the facultative anaerobic reduction of NO_3^- or NO_2^- to NO, N_2O or N_2 (*Bremner*, 1997). This reductive pathway provides electrons for microbial respiration under conditions with O_2 depletion using N oxides as terminal electron acceptors (*Giles et al.*, 2012). However, some of the reductive N transformation steps may also occur under aerobic soil conditions with N_2O as the main product (*Giles et al.*, 2012; *Morley et al.*, 2008). Beside the availability of N oxides function as substrate, the supply of easily available carbon (C) or other electron donators is essential. During denitrification,

heterotrophic denitrifiers using organic C compounds as electron donator through oxidation as well as for metabolic assimilation (*Giles et al.*, 2012). In contrast to autotrophic denitrifiers which might use reduction equivalents such as sulfuric compounds (*Oh et al.*, 2001).

Typical representatives for denitrifiers in soils are *e.g.* bacteria of the genus *Pseudomonas*, *Thiobacillus denitrificans*, *Corynebacterium*, *Paracoccus*, *Chromobacterium*, *Hyphomicrobium* and *Serratina* (*Ingraham*, 1981). However, various types of fungi, such as *Fusarium*, *Acremonium* and *Aspergillus* are also able to reduce N oxides in the same way (*Malinowski and Ottow*, 1985).

The main key factors influencing denitrification in soils are O_2 partial pressure, NO_3^- concentration, availability of reduction equivalents (C) and soil pH (*Robertson*, 1989). Whether N_2O or N_2 is the dominant end product of denitrification depends on these key factors and further biotic and abiotic factors (Figure 2.4).

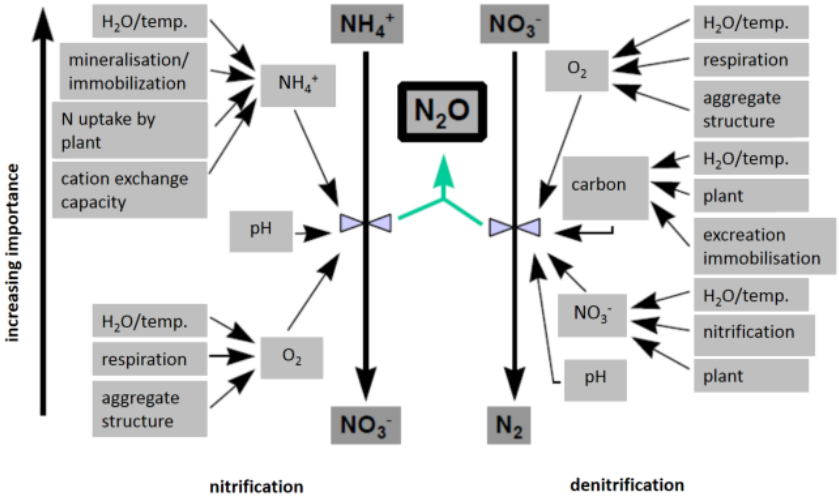

Figure 2.4: Factors influencing N_2O, NO and N_2 forming processes (*Schmädeke*, 1998).

O_2 partial pressure in soils is mainly affected by two factors, O_2 diffusion into the soil and the respiratory O_2 consumption. O_2 diffusion is influenced by, *e.g.* soil moisture level or soil structure (bulk density, soil texture, pore structure) (*Robertson*, 1989; *Smith*, 1990; *Weier et al.*, 1993). Soil respiration depends on temperature and might be stimulated through the addition of substrate for microbial degradation (*Lloyd and Taylor*, 1994; *Pfab*, 2011). Therefore, especially fresh organic matter (OM) with low C/N ratio or quality which is easy to metabolize by microbes might create microsites of high soil respiration (*Flessa and Beese*, 2000; *Garcia-Ruiz and Baggs*, 2007). In principal, the N_2/N_2O ratio increases with decreasing O_2 availability due to the general abatement of terminal electron acceptors (NO_3^-, N_2O) for denitrification (*Giles et al.*, 2012; *Weier et al.*, 1993). In contrast, under conditions with higher redox potential, N_2O is the dominant

end product of denitrification (*Weier et al.*, 1993). *Morley et al.* (2008) attributed this observation to the high O_2 sensitivity of the N_2O reductase.

The NO_3^- content in soils is mainly affected by N inputs (*e.g.* mineral or organic fertilizers applied), N outputs (*e.g.* plant uptake, N removal, gaseous or leaching losses) and by N transformation processes affecting NO_3^- formation/ availability (mineralization, immobilization, nitrification) (*Hart et al.*, 1994).

When the NO_3^- concentration is low, N_2O is reduced more frequently to N_2 due to the competitive effect of NO_3^- and N_2O function as electron acceptor during the process of denitrification (*Cho and Sakdinan*, 1978; *Ruser and Schulz*, 2015). Furthermore, due to the lower energy yield gained from N_2O reduction in comparison to other N oxides prior in the reduction chain of denitrification, the complete reduction to N_2 instead of N_2O is higher under soil conditions with lower NO_3^- concentrations (*Koike and Hattori*, 1975).

How denitrification is stimulated by easily available C depends on quantity and quality of the C compounds (*Morley and Baggs*, 2010; *Pfab*, 2011). The microbial availability of organic C can increase denitrification rates directly by providing the essential reductants or indirectly through the creation of anaerobic soil conditions by increasing microbial respiration during degradation of the organic substrate (*Azam et al.*, 2002; *Giles et al.*, 2012). As reported repeatedly, the addition of OM, such as slurry, green manure, falling crop leaves or harvest residues might increase the supply of easily available organic C in the short-term (*Alluvione et al.*, 2010; *Chantigny et al.*, 2001). Although the response of denitrification is always context specific, the C form and quantity might have an immense influence (*Giles et al.*, 2012). If the C amount is the limited factor for denitrification, it was observed repeatedly that the N_2/N_2O ratio decreased due to an incomplete reduction of the N oxides (*Bouwman*, 1990; *Weier et al.*, 1993). In contrast, an adequate amount of organic reductants increases the N_2/N_2O ratio lowering GHG release (*Firestone and Davidson*, 1989; *Giles et al.*, 2012; *Weier et al.*, 1993). With regard to quality, C traits can affect both, denitrification rates and the C amount necessary to reduce a certain amount of NO_3^- (*Giles et al.*, 2012). In principal, negative correlations between C/N ratios of the organic substrates added and N_2O emissions were reported (*Aulakh et al.*, 2001; *Baggs et al.*, 2000; *Wrage et al.*, 2004). Under soil conditions with low N level, the addition of OM with C/N ratios above 40 could result in net NO_3^- immobilization reducing denitrification rates (*Vigil and Kissel*, 1991). Beside C/N ratio, the carbon's quality is also affected by secondary plant substances from harvest residues (*Pfab*, 2011) or plant roots (*Henry et al.*, 2008) which may also have an effect on denitrification rates. Moreover, biotic factors (*e.g.* substrate-specific alteration of microbial community) or abiotic factors (*e.g.* soil O_2 partial pressure) may also influence the carbon's impact on denitrification (*Giles et al.*, 2012).

Under aerobic soil conditions, the denitrifying N_2O formation might occur through the creation of microsites with high microbial activity by *e.g.* incorporating OM easily mineralized (*Flessa and Beese*, 2000, 1995; *Parkin*, 1987). Through mineralization and nitrification of the organic substances in aerobic soil zones, NO_3^- supply for denitrification in anaerobic soil zones is secured. Due to the high mineralization rates in aerobic soil zones, the amount of electron acceptors increases. Thus, microsites of high denitrification activity might exceed denitrification rates under total anaerobic soil conditions (*Abou Seada and Ottow*, 1985; *Parkin*, 1987).

The optimal pH for denitrification is in the neutral to alkaline pH range (*Wijler and Delwiche*, 1954). Numerous enzymes are involved in the process of denitrification (Figure 2.3). The N_2O reductase (*nosZ*), the enzyme for the last reduction step, is the most pH, O_2 and temperature sensitive enzyme within the whole denitrifying reaction chain, inhibited by acidic soil conditions (*Hu et al.*, 2015), aerobic conditions (*Morley et al.*, 2008) or soil temperatures below 5 °C (*Mørkved et al.*, 2006).

2.2.2.2 Nitrification

Nitrification is the microbial oxidation of NH_3 over hydroxylamine (NH_2OH) and NO_2^- to NO_3^-. During nitrification, the gas N_2O may be produced during oxidation of NH_2OH. Nitrification is an aerobic two-step process. During the first step of reaction, the ammonia monooxygenase (AMO) oxidize NH_3 to NH_2OH before the hydroxylamine oxidoreductase (HAO) converts NH_2OH to NO_2^- (Figure 2.3). Typical representatives of nitrifiers for the first step of reaction are obligate aerobic bacteria of the genus *Nitrosomonas*, *Nitrosococcus*, *Nitrospira* and *Nitrosolobus* (*Blackmer et al.*, 1980; *Bremner and Blackmer*, 1981) or NH_3 oxidizing archaeas of the genus *Nitrosopumilus* or *Nitrososphaera* (*Fuchs and Schlegel*, 2014).

During the second step of nitrification, NO_2^- is oxidized to NO_3^- through bacteria of the genus *Nitrobacter* or *Nitrospina* (*Fuchs and Schlegel*, 2014). In general, the second step of nitrification runs faster than the first step. Thus, NO_2^- in soils is only detectable in traces (*Giles et al.*, 2012). There are two possibilities for N_2O formation during nitrification. Firstly, it can be produced as byproduct during NH_3 oxidation through spontaneous decomposition of NH_2OH to N_2O (Figure 2.3). Secondly, N_2O might be formed through the reduction of NO_2^- during the process called nitrifier-denitrification (Figure 2.3). Generally, the first pathway has greater importance for N_2O production (*Arp and Stein*, 2003; *Butterbach-Bahl et al.*, 2013). However, under soil conditions with increasing O_2 depletion, the process of nitrifier-denitrification could gain crucial relevance (*Groffman*, 1991; *Wrage et al.*, 2004). Here, anaerobic soil conditions limit NO_2^- oxidation. Thus, autotroph nitrifying bacteria are using NO_2^- as electron acceptor reducing it to N_2O or possibly to N_2 (*Butterbach-Bahl et al.*, 2013). Thus, the accumulation of the cytotoxic NO_2^- is similarly prevented (*Firestone and Davidson*, 1989). In general, less

than 1 % of the N nitrified is released as N_2O-N (*Firestone and Davidson*, 1989). *Flessa et al.* (1996) just detected 0.06 % of the fertilized NH_4^+-N in the form of N_2O-N.

Numerous factors influence nitrification directly or indirectly (Figure 2.4). The NH_4^+ content in soil solution as substrate for nitrification determines the amount of N nitrified. The optimum pH for nitrification is in the slightly alkaline range (*Kuntze et al.*, 1994). The generally accepted explanation for delayed nitrification rates under acidic soil conditions is attributed to the AMO which prefers NH_3 instead of NH_4^+ for oxidation (*Nicol et al.*, 2008; *Suzuki et al.*, 1974).

Optimum soil temperature for autotrophic nitrification ranges between 15 and 35 °C (*Kuntze et al.*, 1994). C/N ratios of the top soil layer of 25 or less stimulate nitrification (*Blume et al.*, 2010). The optimal soil moisture ranges around field capacity. High soil moisture contents bear the risk of inhibiting nitrification through O_2 depletion whereas low soil moisture statuses reduce nitrification rate through limiting microbial activity (*Subbarao et al.*, 2006).

2.2.3 Environmental factors regulating N_2O releases

Numerous factors affect N_2O formation (Chapter 2.2.2). Thus, management factors, such as fertilizer form provide opportunities to intervene in N_2O release or consumption in arable soils.

Numerous studies have reported that manure application results in higher N_2O emission than the application of mineral fertilizers (*Flessa and Beese*, 2000; *Kaiser and Ruser*, 2000; *Velthof and Mosquera*, 2011). Thereby, the height of N_2O emissions might depend on manure type and quality (*Velthof et al.*, 2003). In contrast to mineral fertilizers, organic N-fertilizers provide easily available C, which is frequently the limiting factor for denitrification in N-fertilized arable soils (*Firestone and Davidson*, 1989; *VanderZaag et al.*, 2011). Beside the supply of reductants by slurry addition, slurry derived C was also reported to stimulate microbial activity through the creation of conditions favorable for denitrification by increasing microbial respiration (*Dosch and Gutser*, 1996; *Flessa and Beese*, 2000). The effect of O_2 depletion after organic fertilization might be reinforced additionally through O_2 consuming processes, such as nitrification of slurry derived NH_4^+–N (*Van Nguyen et al.*, 2017).

Due to the different properties of organic and mineral N-fertilizers, N_2O mitigation strategies approached for synthetic N-fertilizers, *e.g.* slow nutrient release through capsulation, may not always be practical for organic fertilizers. In Table 2.2, N_2O mitigation strategies after manure application are listed. According to *de Klein and Ledgard* (2005), the general approaches for N_2O reduction after organic fertilization are the reduction of N input (manure N applied) and reduction of N losses (increase nitrogen use efficiency

(NUE) of applied manure-N and avoiding soil conditions favorable for direct or indirect N_2O formation).

Table 2.2: Strategies to mitigate N_2O emissions from land applied manure (*Gutser et al.*, 2000; *Ruser et al.*, 2016; *Subbarao et al.*, 2006; *VanderZaag et al.*, 2011; *Webb et al.*, 2010).

Dietary measures	Reduce excretion N or change manure composition by reducing N content in diet
Manure separation	Increase O_2 availability and delay N mineralization after application through reducing manure's moisture content and labile C content by separation
Application technique	On arable land, surface application with incorporation is the most appropriate application technique. Slurry injection is exclusively recommendable under dry soil conditions (Chapter 3) N_2O emissions are reduced with increasing incorporation depth due to increasing diffusion path On grassland, trailing shoe is preferable
Application timing	Soil conditions should not promote nitrification (*e.g.* high temperature) or denitrification (*e.g.* high moisture) pre and post application Application timing depending on crop development
Application rate	N_2O emissions increase with rising application rate, thus, splitting of the organic fertilizer might be conceivable Fertilizer rate should be adapted to plant demand in time and space
Amendments	Nitrification inhibitors (NIs) (Chapter 2.5) N_2O reduction through delayed mineralization/ increased N-immobilization through enhancing C/N ratio of manures by adding OM rich in C (*e.g.* woody compost) Liming: increased pH reduces N_2O release through optimizing conditions for nitrification (reduce N_2O formation) or denitrification (enhance conversion of N_2O to N_2)
Crops	Increase NUE by using genotypes with improved N uptake Cover crops can reduce N_2O emissions indirectly by reduce NO_3^- leaching Remove crop residue with low C/N ratio from the field
(no-) tillage	Improve soil structure through optimize aeration and drainage (*e.g.* reduced soil compaction)

2.3 Ammonia

NH_3 losses contribute to eutrophication and acidification of environmentally sensitive areas (*Fangmeier et al.*, 1994; *Houdijk and Roelofs*, 1991) and natural water resources (*Sutton and Fowler*, 2002). Moreover, NH_3 is an indirect GHG because it induces the formation of N_2O in non-agricultural ecosystems after deposition (*Nevison*, 2000). *Bouwman et al.* (1997) calculated global anthropogenic NH_3 emissions of 34 Tg N yr^{-1} with excreta from domestic animals as the dominant NH_3 source (21.6 Tg N yr^{-1}). The *IPCC* (2006) default value, assume that 1 % of NH_3–N deposited is re-emitted as N_2O-N. In Germany, the agricultural sector is also the main source for NH_3 emissions with a share of approximately 95 % on the total annual NH_3 emissions. Thereby, livestock keeping is the major contributor. In each step of animal manure management (housing,

storage, application, grazing) several factors affect NH_3 losses (*Rösemann et al.*, 2017). In Germany, the highest amounts lost as NH_3 for the whole animal agriculture originate from application of organic fertilizers (43.4 %), followed by housing (39.4 %), storage (15.5 %) and grazing (1.7 %) (*Rösemann et al.*, 2017).

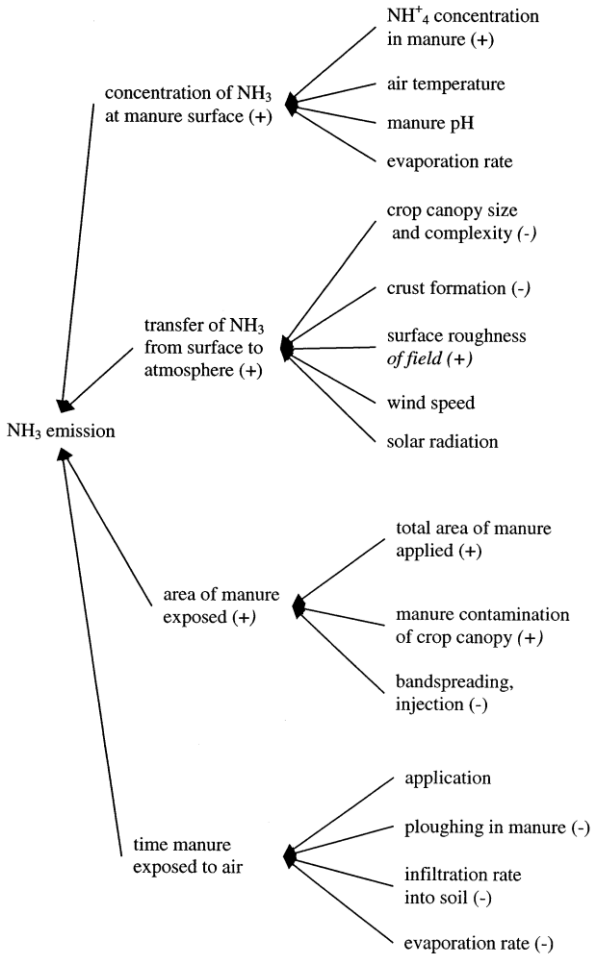

Figure 2.5: Factors controlling NH_3 volatilization from field-applied manures (*Sommer and Hutchings*, 2001).

One primary reason, influencing NH_3 emissions after spreading, are manure properties. Factors, like ammoniacal nitrogen (TAN), pH value and dry matter (DM) of animal manures strongly affect the manure based NH_3 emissions after application (*Sommer and Hutchings*, 2001). NH_3 losses were shown to be extremely weather dependent (Figure 2.5). Particularly a high wind velocity, solar radiation and air temperature increase NH_3 emission (*Horlacher and Marschner*, 1990; *Sommer et al.*, 2003). Moreover, NH_3 emissions generally increase with rising time and exchange area between slurry and the atmosphere. Both are related to the application technique. *Sommer and Hutchings* (2001) suggested slurry injection or incorporation as the most efficient measure to reduce NH_3 emissions from field applied manures (Figure 2.6).

However, further mitigation strategies might also reach a substantial reduction of field-related NH_3 emissions (Table 2.3).

Table 2.3: Management factors decreasing NH_3 losses (*Sommer and Hutchings*, 2001).

Weather	Slurry application on a cloudy, cool and windless day
Infiltration	Increasing slurry infiltration through application before rain, irrigation, cultivation
	Decreasing viscosity/ DM of the slurry
pH	Reduce slurry's pH to below 6 through acidification
Application technique	Reduce surface area of the slurry by injection, incorporation
	For plant covered soil use banded application by trail hose
Timing	Start slurry incorporation as soon as possible after surface application
	Carry out slurry application at the coolest part of the day (*e.g.* in the evening)

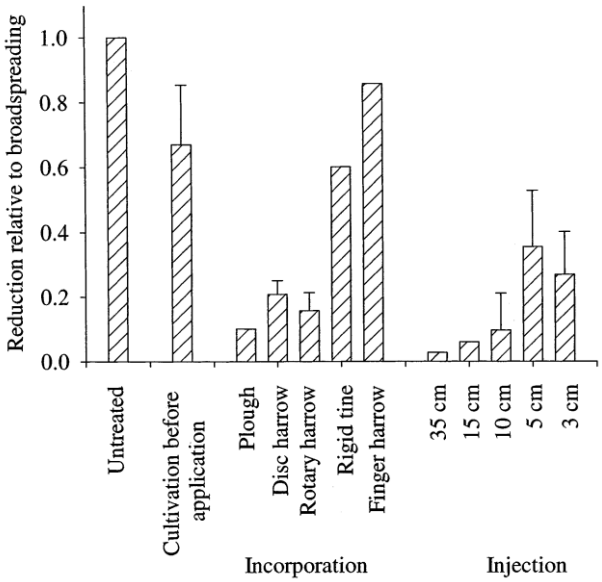

Figure 2.6: Effect of application technique and injection depth on NH_3 emissions after animal slurry amendment onto soil or a plant covered soil (*Sommer and Hutchings*, 2001).

2.4 Methane

2.4.1 Methane and climate change

In spite of its short atmospheric lifetime (10 years), CH_4 is the second most important anthropogenic trace gas contributing to greenhouse effect with a share of 16 % (*IPCC*, 2014b). Its global warming potential (GWP) of 23 is primary attributed to absorption capacity of infrared radiation (*European Commission*, 2010; *Le Mer and Roger*, 2001). Since 1750, the atmospheric CH_4 concentration increased by 150 % resulting in atmospheric CH_4 concentration of about 1800 ppb nowadays (*Blasing*, 2014; *Myhre et al.*, 2013). This increase is caused by a rising imbalance between CH_4 sources and sinks

strength. The majority of CH_4 emissions are formed biologically by methanogenic microbes during anaerobic digestion of OM (methanogenesis). According to *Le Mer and Roger* (2001), about 70 % of emissions can be traced back to anthropogenic activities. Thereby, CH_4 emissions from agricultural sources, such as enteric fermentation by ruminants or paddy rice cultivation are the main contributors (*Denman et al.*, 2007).

In contrast to biological CH_4 production, the major CH_4 sink is the photochemical degradation by hydroxyl ($\cdot OH$) radicals through oxidation in the troposphere (*Le Mer and Roger*, 2001). However, biological CH_4 oxidation in terrestrial ecosystems also reduces atmospheric CH_4 concentration contributing approximately 5 % of the annual global CH_4 sinks (Table 2.4).

Table 2.4: Sources and sinks of CH_4 (*Denman et al.*, 2007).

	Range [Tg CH_4 yr^{-1}]
CH_4 sources (in total)	**503 – 610**
Anthropogenic sources	**264 – 428**
Energy	74 – 77
Coal mining	30 – 48
Gas, oil, industry	36 – 68
Landfills and waste	35 – 69
Ruminants	76 – 189
Rice agriculture	31 – 112
Biomass burning	14 – 88
C_3 vegetation	27
C_4 vegetation	9
Natural sources	**145 – 260**
Wetlands	100 – 231
Termites	20 – 29
Ocean	4 – 15
Hydrates	4 – 5
Geological sources	4 – 14
Wild animals	15
Wildfires	2 – 5
CH_4 sinks (in total)	**492 - 581**
Soils	26 – 30
Tropospheric OH	445 – 511
Stratospheric loss	30 – 45

2.4.2 Processes of microbial CH_4 dynamics in soils

As shown in Table 2.4, soils can contribute to global CH_4 budget as CH_4 source or sink. Thus, enzymatic CH_4 oxidation or biological CH_4 production are of particular importance in the global CH_4 cycle.

In soil zones or other environments under anaerobic conditions, CH_4 is produced by methanogenesis whereas in well-aerated compartments, CH_4 concentrations are reduced

through CH_4 oxidation to CO_2. Whether an environment is a net source or sink depends on the balance between these two processes (*Bédard and Knowles*, 1989).

2.4.2.1 Methanotrophy

Biological CH_4 consumption by methanotrophic bacteria (methanotrophs) is used for energy production and substrate supply (*Bédard and Knowles*, 1989). In soils, two forms of methanotrophs are recognized, one with high affinity oxidation occurring at atmospheric CH_4 concentrations less than 12 ppm and another form, known as low affinity oxidation occurring under CH_4 concentrations above 40 ppm being typical for paddy rice soils (*Le Mer and Roger*, 2001). Thereby, the methane monooxygenase (MMO) is the key enzyme of these oxidation processes, requiring the availability of O_2 and CH_4 (*Hanson et al.*, 1996). A fairly similar enzymatic oxidation process is the oxidation of NH_3 to NH_2OH via the AMO (Figure 2.7). Due to a related molecular structure, both enzymes are able to oxidize NH_3 and NH_4^+ (*Holmes et al.*, 1995). Under conditions with high NH_4^+ availability, *e.g.* after fertilization, a reduced CH_4 oxidation was reported repeatedly (*Bronson and Mosier*, 1994; *Conrad and Rothfuss*, 1991; *Dunfield and Knowles*, 1995; *Hansen et al.*, 1993). *Bédard and Knowles* (1989) attributed the reduced CH_4 oxidation rates to competitive inhibitory effect of MMO initialized by NH_4^+ which competes with CH_4 for binding site.

When NH_4^+ (and/or NH_3) binds to MMO, the enzymatic pathway of methanotrophic nitrification runs until the oxidation of NH_2OH to NO_2^- as end product. According to *Jollie and Lipscomb* (1991) and *Schnell and King* (1994) the increased NO_2^- concentrations have a toxic effect on microbes and probably inhibit the formate dehydrogenase (Figure 2.7).

Thus, the NH_4^+ -induced reduction in CH_4 consumption is reinforced by methanotrophic nitrification (*Topp and Pattey*, 1997). Beside the presence of NH_4^+, numerous factors control the height of CH_4 oxidation in soils. *Schimel et al.* (1993) listed the main influencing factors for CH_4 oxidation hierarchically. Thereby, gas diffusion into the soil was characterized as the primary driver controlling CH_4 oxidation. The soil aeration is associated closely with several physical properties, such as water content, soil texture, air-filled porosity or tortuosity. Thus, changes in soil moisture (*e.g.* through precipitation/ irrigation, transpiration, groundwater level) or soil structure (*e.g.* through bioturbation, tillage) may have an effect on diffusion rates. Due to the dominant influence of physical properties and the complex relationships affecting CH_4 consumption, the influence of chemical or biological soil properties which have a minor impact might be masked (*Schmädeke*, 1998). For example, soil temperature affects biological activity of methanotrophs but also affects evapotranspiration and thus has an impact on gas diffusion rates which might cover the biological effect. Equally, it is difficult to determine an

optimum soil pH for CH_4 oxidation. Under soil conditions with low pH, a reduced activity of methanotrophs was observed repeatedly (*Borken and Brumme*, 1997; *Dunfield et al.*, 1993).

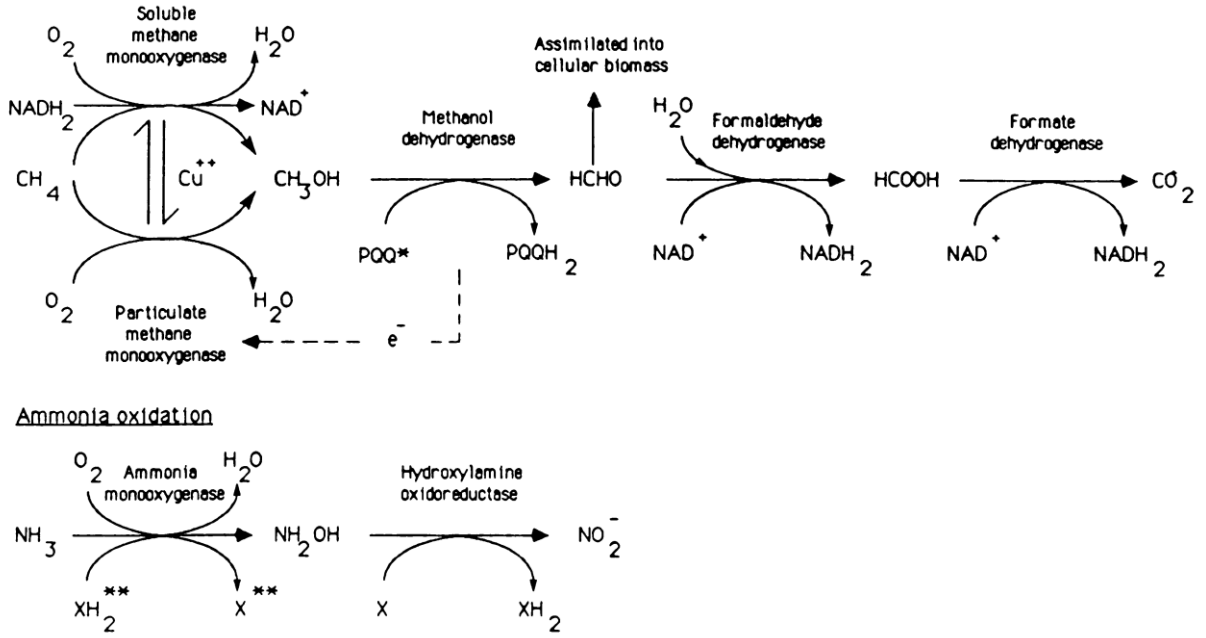

Figure 2.7: Pathway of methane oxidation in methanotrophs and ammonia oxidation in ammonia oxidizers. *PQQ Pyrroquinoline quinone, **X and XH_2 are the oxidized and reduced forms of an unknown electron donor (*Bédard and Knowles*, 1989).

However, the reduced oxidation rates might be caused by enhanced presence of NH_4^+ through inhibited nitrification under acidic soil conditions (*Tate*, 2015).

2.4.2.2 Methanogenesis

In Europe, agricultural soils are mainly well-aerated terrestrial ecosystems with net CH_4 uptakes (*Hütsch*, 2001). However, anoxic conditions with redox potentials of -150 mV or less favoring methanogenesis can occur under waterlogged soil conditions or through increased microbial respiration (*Masscheleyn et al.*, 1993; *Schimel et al.*, 1993).

Methanogenesis completes the last step in anaerobic fermentation process of OM degradation occurring when electron acceptors such as O_2, NO_3^-, ferric (Fe^{3+}), sulfate (SO_4^{2-}) were already reduced (*Schimel et al.*, 1993; *Topp and Pattey*, 1997). Thereby, methanogenic microorganisms (methanogens) are highly substrate specific using exclusively simple methanogenic substrates, such as atomic hydrogen (H_2) + CO_2 or acetate (CH_3COOH).

Under appropriate redox conditions, the primary factor controlling methanogenesis is the supply of organic C compounds available for methanogens (Figure 2.8). As a result of this metabolic specificity, the prior degradation of complex molecules is crucial for CH_4 formation. Consequently, methanogens are dependent on activities of the microbial

communities responsible for substrate provision (*Topp and Pattey*, 1997). Furthermore, factors like low soil temperature might limit methanogenesis rather through decreased degradation rates than methanogenesis itself (*Schimel et al.*, 1993). Nevertheless, optimum soil temperatures for methanogenesis range from 30 to 40 °C (*Conrad et al.*, 1987). In contrast to factors limiting CH_4 formation, the addition of OM rich in easily mineralizable C might increase the availability of small C molecules and enhance microbial respiration, both stimulating methanogenesis (*Le Mer and Roger*, 2001) (Figure 2.8). In comparison to methanotrophs, methanogens are more temperature and pH sensitive. The optimum soil pH for methanogenesis is around the neutral or slightly alkaline range (*Le Mer and Roger*, 2001; *Schimel et al.*, 1993). Further factors controlling CH_4 production are listed hierarchically in Figure 2.8.

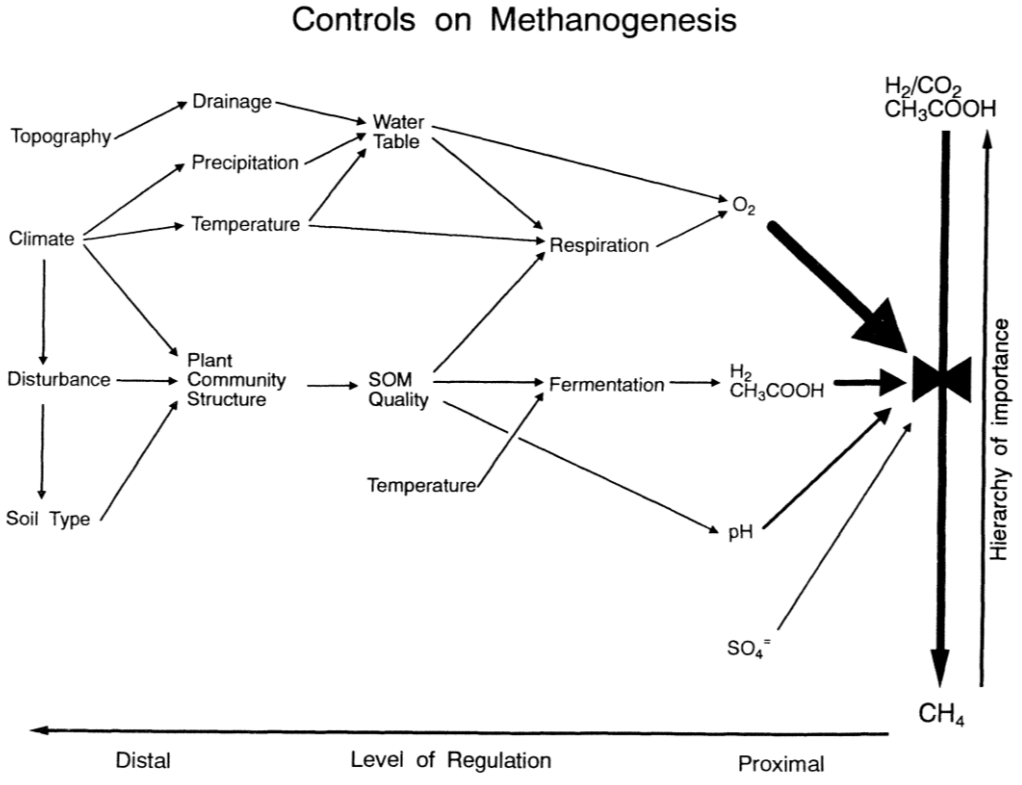

Figure 2.8: Relationship between proximal and distal controls on CH_4 production in anaerobic soils (*Schimel et al.*, 1993).

How CH_4 oxidation and CH_4 production might be affected by management factors in generally well-aerated arable soils is discussed in Chapter 5.

2.5 Nitrification inhibitors (NIs)

According to *Ruser and Schulz* (2015), NIs are an efficient tool to reduce N_2O emissions and NO_3^- leaching losses from arable soils enabling an increase in NUE of cropping systems. Most commercial NIs inhibit the enzymatic activity of AMO, the first enzyme

involved in nitrification (oxidation of NH_3 to NH_2OH, refer to Figure 2.7) (*Subbarao et al.*, 2006).

Inhibiting the first step of NH_3 oxidation is a very efficient way of reducing N_2O emissions since this intervention blocks all further enzymatic N_2O formation processes directly or indirectly (refer to Figure 2.3) without risking an accumulation of NH_2OH which might have toxic effects on *Nitrosomonas* (*Arp and Stein*, 2003). NIs directly reduce nitrification rates and thus NO_3^- concentration in soil which might serve as substrate for denitrification. Consequently, the two main pathways responsible for N_2O formation in soils are reduced greatly through NI addition (*Bremner and Yeomans*, 1986; *Ruser and Schulz*, 2015). Furthermore, a lower NO_3^- concentration stimulates denitrifying microorganisms to reduce N_2O and shifts the N_2/N_2O ratio towards higher N_2 portions (*Blackmer and Bremner*, 1978; *Cho and Sakdinan*, 1978; *Ruser et al.*, 2006). Thus, N_2O emissions attributed to denitrification are further decreased indirectly whereby the effect strength of NIs concerning atmospheric burden is enhanced.

Beside N_2O mitigation, the NI-induced NH_4^+ conservation might also prevent NO_3^- leaching losses. Due to the increased risk of NO_3^- leaching and a strong reduction of plant-available N, this is of particular importance especially for coarse-textured soils with low SOM contents in combination with heavy rainfall events or irrigation (*Gaines and Gaines*, 1994; *Pfab et al.*, 2012). Through the addition of NIs, several studies confirmed significantly reduced leaching losses from fertilizer N resulting in improved NUE and increased economic yields (*Fangueiro et al.*, 2009; *Federolf et al.*, 2016; *Prasad and Power*, 1995; *Ruser and Schulz*, 2015; *Sutton et al.*, 1986).

2.5.1 Mode of action

Different mechanisms have been described inhibiting the key enzyme AMO itself or influencing its enzymatic activity. The mode of actions of approved NIs are categorized in the following groups (*Ruser and Schulz*, 2015):

(1) Direct binding and interaction with AMO (*Keener and Arp*, 1993). The inhibition of AMO can be implemented by competitive or non-competitive inhibition. If the active substance resembles NH_3 and competes to the active binding site of AMO, it is a competitive inhibition. If the added compound binds to a second site of AMO, which is not used for NH_3 binding, and changes the enzymatic formation, the substrate binding in the active site is blocked or/and the catalytic process is inhibited, it is a non-competitive inhibition (*Campbell and Reece*, 2009). *Keener and Arp* (1993) assumed, that the binding site for non-competitive inhibition in *Nitrosomonas* ssp. is non-polar due to the inhibitory effect increased with enhanced molecular size of hydrophobic alkanes and halogenated hydrocarbons.

(2) Removal of co-factors by chelating compounds (*Ruser and Schulz*, 2015). The AMO is localized in the membrane of microorganisms with copper (Cu) as co-

factor (*Arp et al.*, 2002). The removal of the co-factor Cu by chelating compounds is therefore able to inhibit the AMO (*Bédard and Knowles*, 1989). *McCarty* (1999) assumed that this inhibitory effect is performed by binding Cu within the active site of AMO.

(3) Mechanism-based inhibitors of enzymes are defined as chemical compounds, which have an irreversible inhibitory effect after the catalytic conversion of the compounds by the appropriate enzyme. Mostly, it results in an irreversible inhibition of AMO, caused by covalently binding of the highly reactive catalyst product and the oxidizing enzyme (*McCarty*, 1999). Some of these highly reactive catalyst products only bind to AMO (*Hyman and Arp*, 1992) whereas others are covalently bound to several cellular components (*Hyman et al.*, 1995; *McCarty*, 1999). In both cases, a *de novo* synthesis of one or several proteins is necessary to neutralize the inhibitory effect. Consequently, the inhibition apparent more persistently, the more proteins need to be *de novo* synthesized (*Hyman et al.*, 1995).

A further important class of NIs are heterocyclic N compounds, whereas the mode of action has not yet been clarified (*McCarty*, 1999). *McCarty and Bremner* (1989) showed, that heterocyclic N compounds containing two or three adjacent ring N atoms, inhibited NH_3 oxidation in soil significantly compared to heterocyclic N compounds with less N atoms or non-adjacent ring N atoms.

Different NIs vary in their mode of action and mostly, it is not possible to classify a NI strictly in one group of inhibitors due to the active compound(s) influence(s) several mode of actions simultaneously (*Ruser and Schulz*, 2015).

2.5.2 General factors influencing NI efficiency

As summarized from *Subbarao et al.* (2006), the effective inhibition of nitrification by NIs in field is related to numerous interactions with physical, chemical and biological factors (Table 2.5). NIs differ in their mobility and persistence in soils due to different chemical characteristics like water solubility and volatility. Therefore, their effectiveness varies under different environmental conditions. Generally, the same mobility in soils as NH_4^+ is comfortable, whereby only a few of NIs have this property (*Subbarao et al.*, 2006). Moreover, NIs which are volatile have no long persistence in soil due to their volatility loss through soil pores. However, they inhibit nitrification more rapidly than inhibitors with a higher persistence (*Ashworth et al.*, 1977).

Beside their chemical characteristics, NIs are strongly affected by environmental conditions, especially by soil factors, which influence their effectiveness differently.

Irigoyen et al. (2003) reported that the soil temperature is the most influencing factor for persistence of NIs in soils. Most studies observed a decreasing stability of NIs with

increasing soil temperatures. At a soil temperature of ≤5 °C, nitrification can be inhibited through additives up to six months (*Slangen and Kerkhoff*, 1984; *Zerulla et al.*, 2001). From soil temperatures above 10 °C, there is a linear decrease of the inhibition effectiveness and at temperatures ≥25 °C, NH_4^+ conservation occurs only two up to three weeks for most of the inhibitors, whereby especially volatile inhibitors are less stable at higher temperatures (*Prasad and Power*, 1995; *Subbarao et al.*, 2006; *Zerulla et al.*, 2001). The negative correlation between soil temperature and inhibitory effect is justified by a decreasing persistence and an increasing biological activity of nitrifiers at higher soil temperatures.

Due to their different chemical characteristics, NIs are differently influenced by soil texture and soil organic matter (SOM) (*Slangen and Kerkhoff*, 1984). In general, it has been reported, that NIs have a lower persistence and effectiveness as finer the soil texture and as higher the SOM content (≥1 %). This is mainly due to the sorption effect and the decomposition rate of NIs (*McClung and Wolf*, 1980). Consequently, sorption of NIs by SOM decreases their mobility, volatility and bioactivity which leads to a reduced inhibitory effect (*Keeney*, 1986). Additionally, volatile inhibitors have a reduced effectiveness as higher the soil porosity.

Commonly, NIs showed a higher inhibition potential under soil moisture contents below field capacity, probably due to a reduced activity of nitrifiers (*Subbarao et al.*, 2006), whereby the water solubility of the NI is decisive. Since the higher the water solubility so too is the greater risk of leaching and subsequently the lower the inhibitory effect after soil rewetting through heavy rainfalls or irrigation (*Ruser and Schulz*, 2015; *Zerulla et al.*, 2001). Furthermore, volatile inhibitors become more unstable in saturated soils due to higher hydrolysis and volatilization (*Hendrickson and Keeney*, 1979; *Slangen and Kerkhoff*, 1984).

The soil pH influences the composition of nitrifying organisms and consequently the effectiveness of NIs indirectly depending on which group of nitrifiers is mainly affected by the inhibitor. Moreover, the chemical stability and efficiency of NIs varies with different soil pH, whereby a pH range from 3 up to 7 is covered by most of the marketable NIs (*Slangen and Kerkhoff*, 1984; *Subbarao et al.*, 2006).

Table 2.5: Factors that determine the effectiveness of nitrification inhibitors in soils (*Subbarao et al.*, 2006).

Inhibitor properties	
Water solubility	Determine the mode of application, leaching of the inhibitor
Volatility	Determine the movement in soil, and also effectiveness at high temperatures
Sorption on colloids (particularly on OM or clay)	Limits the rate of leaching and mobility in soil
Stability (rate of degradation)	Persistence of the inhibitor in soils
Soil chemical and physical properties	
pH	Affects stability and solubility of the inhibitor; also affects nitrifier activity upon which the inhibitor is expected to show its affect
OM levels	Sorption of the inhibiting compound, affects mobility and stability of the inhibiting compounds
Porosity	Affects O_2 levels in soils which determine the nitrifier activity; also, determine the effectiveness of inhibitors that have high volatility
Soil N from mineralization of OM	NH_4^+ -N produced through soil mineralization may not be accessable to the inhibitor to prevent nitrification
Soil biological properties	
Nitrifier population	The biological activity of the nitrifier population will also determine the effectiveness of the inhibitor; in soils, that have very high rates of nitrification, higher inhibitor concentrations are needed to control; also, genetic diversity of *Nitrosomonas* strains, which may vary their sensitivity to the nitrifiers thus can modulate the inhibitory effect depending on the genetic make up of the nitrifier population in a given region.
Soil C levels	May determine the heterotrophic microbial activity in soils, will influence the rate of NH_4^+ microbial immobilization. Also, heterotrophic microbial populations may metabolize, thus decompose the inhibitor compounds, and limit their persistence in soils.
Abiotic factors	
Temperature	Nitrifier activity is temperature dependent, lower temperatures (such as winter season) usually have low nitrifier activity, thus inhibitors are effective; as the soil temperatures increase to above 15 °C and more (usually spring and summer), nitrifier activity will increase, thus more difficult for the inhibitors to control nitrification. Also, many inhibitors are physically and biologically unstable at temperatures of 15 °C and above, their effectiveness decreases linearly with an increase in temperature.
Water status	Determines the nitrifier activity and the movement of inhibitor in the soil. It also affects aeration.
Other factors related to fertilizer type and mode of application	
Type of fertilizer (*i.e.*, ammonium sulfate or urea)	Fertilizer influence soil pH as ammonium sulfate usually results in acidic pH and urea-N results in alkaline pH.
Mode of application (*i.e.*, banding vs. broadcasting)	NIs application as a band on to the banded fertilizer is usually more effective than when the inhibitor is broadcasted along with the fertilizer, but requires higher concentrations of the inhibitor.

2.5.3 NIs as strategy for N_2O mitigation

As mentioned above, due to the number of chemical, physical and biological factors influencing the inhibitory effectiveness of the particular NI differently, a wide range of N_2O reduction potentials were reported. However, numerous studies have shown consistently, that NIs can reduce N_2O emissions from organic or mineral fertilization (*Ruser and Schulz*, 2015). However, the height of N_2O emissions reported after fertilization as well as the reduction potentials though NI additions varied strongly due to differing experimental conditions. In a review from *Ruser and Schulz* (2015), the effectiveness of several NIs was evaluated using data collections from previous studies. Thereby, the authors expanded the dataset from the meta-analysis from *Akiyama et al.* (2010) from 85 to 140 studies. Due to the wide range of influencing factors, the summary of these results gives no universally valid conclusion, but might be taken as an indicator. *Ruser and Schulz* (2015) reported the highest potential of reducing N_2O emissions for 1,2,4 Triazole and 3-Methylpyrazole (TZ & MP) with 55 % compared to untreated fertilizer (*n*=2), followed by dicyandiamide (DCD) with 40 % (*n*=79), 3,4-dimethylpyrazole phosphate (DMPP) 34 % (*n*=17), Nitrapyrin 32 % (*n*=12) and 3,4-dimethylpyrazole succinic acid (DMPSA) 28 % (*n*=1). Unfortunately, due to the generally rare dataset of animal manure application together with NIs, most of the included studies used mineral fertilizers. Consequently, the effect of different NIs applied with organic N-fertilizers needs to be evaluated in further studies.

2.5.4 Characterization of marketable NIs

2.5.4.1 3,4-dimethylpyrazole phosphate (DMPP)

*Wissemeier (*pers. comm. in *Ruser and Schulz*, 2015) assumed, that the mode of action of DMPP, which belongs to heterocyclic N compounds with two adjacent ring N atoms, is referred to Cu-chelating compounds. DMPP, commercially known as ENTEC®, was intensively used and tested in agriculture over the last decades and has been confirmed as environmentally safe by toxicological and eco-toxicological test (*Andreae*, 1999; *Roll*, 1999). Moreover, no negative effects on cultivated plants were detected (*Zerulla et al.*, 2001). Thus, DMPP turned out as an appropriate NI, which improves economic and ecological requirements by reducing NO_3^- leaching losses and N_2O emissions from mineral fertilizers strongly with the consequence of improving biomass and grain yields (*Subbarao et al.*, 2006; *Weiske et al.*, 2001).

A novel formula of DMPP markets under the trade name ENTEC® FL containing 25-50 % ammonium nitrate and 1-5 % of the active substance DMPP. ENTEC® FL is a liquid formulation and enables the use of DMPP in slurry and digestates (*EuroChem Agro*, 2014). When compared to other NIs, DMPP has the same mobility in soils as NH_4^+ preventing the separation of these two compounds. This great benefit results in stronger inhibition of NH_3 oxidation (*McCarty*, 1999; *Prasad and Power*, 1995; *Serna*

et al., 2000; *Yu et al.*, 2007; *Zerulla et al.*, 2001). Based on the results from *Azam et al.* (2001), *Ruser and Schulz* (2015) concluded that a spatial separation of DMPP and N fertilized after application depends on soil texture and humus content. They assumed that the separation of NH_4^+ and DMPP might be faster as lower the clay content due to reduced absorption potential for cations. Additionally, they expected an accelerated separation of these two compounds as lower the amount of clay-humus-complexes because of a higher availability of sorption places on clay minerals or organic molecules, which leads to a retarded diffusion of DMPP.
However, the most influencing factor on the length of nitrification inhibition through NIs is the soil temperature (*Irigoyen et al.*, 2003). An incubation study from *Zerulla et al.* (2001) on a loess loam without plants has shown, that at 5 °C DMPP inhibited nitrification over 140 days where nitrification of added NH_4^+ from the control treatment without DMPP was completed within this time frame. At warmer soil conditions of 20 °C the nitrification was completed within 7-21 days from the control without NI and within 40 days with DMPP. *Chen et al.* (2010) reported similar results regarding NH_4^+ conservation from an incubation experiment on a clay loam soil with or without DMPP. Additionally, they detected N_2O emissions and observed a significant reduction of N_2O emissions through DMPP compared to fertilized control treatment under different soil temperatures (5 °C, 15 °C and 25 °C) and soil moisture contents (40 % and 60 % water filled pore space (WFPS)). *Dittert et al.* (2001) observed N_2O emissions after cattle slurry injection with and without DMPP over 22 days. Within this time period, DMPP reduced cumulative N_2O emissions by 32 % compared to the positive control.

The results of 136 field studies under different climatic conditions and cropping systems confirmed that DMPP may increase crop yield for several agricultural crops and enhance the mean maize grain yield by 0.24 Mg ha^{-1} (*Pasda et al.*, 2001). Generally, a stronger yield increase through DMPP addition was observed as lighter the soil texture, as higher the precipitation rate and consequently as higher the risk of NO_3^- leaching losses.

Fangueiro et al. (2009) also attributed a higher inhibitory effect to DMPP compared to DCD when added to cattle slurry under ryegrass cultivation. NH_4^+ conservation lasted 60 days longer in the DMPP treatment and resulted in higher DM yield of ryegrass. On the contrary, *Weiske et al.* (2001) detected no effect of these two NIs concerning yield of summer barley, maize and winter wheat compared to a sole ammonium sulfate nitrate fertilization in a three year field experiment. Based on meta-analyses, *Yang et al.* (2016) observed a higher DMPP efficiency in soils with neutral pH.

2.5.4.2 3,4-dimethylpyrazole succinic acid (DMPSA)

The drastic decrease of effective inhibition with rising soil temperatures demonstrates the potential for optimizing NIs by creating them more persistent. The new NI DMPSA

was developed to combine the generally known inhibitory effect of DMP with the release behavior of the organic acid succinic acid. Therefore, a microbial degradation of succinic acid must be preceded before the active inhibiting substance is released and should result in a delayed and prolonged inhibitory effect (*Pacholski et al.*, 2016). Due to the fact, that the reactive compound of this novel NI is similar to DMPP, it can be assumed that both exhibit the same mode of action.

Up to now, only a few studies tested the effect of DMPSA regarding N_2O emissions, N_{min} dynamics in soil, NUE and yield. However, DMPSA proved to have a delaying effect on nitrification and reduced N_2O emissions in combination with mineral fertilizers (*Pacholski et al.*, 2016). The addition of DMPSA to urea or calcium ammonium nitrate (CAN) reduced N_2O emissions in a field study and an incubation experiment by between 60 % and 90 % in comparison to untreated fertilizer (*Pacholski et al.*, 2016). Similarly, *Guardia et al.* (2018) detected a delayed nitrification and a significant reduction of N_2O emissions through DMPSA addition to ammonium nitrate compared to fertilization without NI during a maize cropping period. In a Spanish field experiment, DMPSA addition reached a significant reduction of yield-scaled N_2O emissions despite no significant increase of maize grain or biomass yields (*Guardia et al.*, 2017; *Pacholski et al.*, 2016). To the best of our knowledge, the effectiveness of DMPSA in combination with organic fertilizers has not yet been tested in previous studies.

2.5.4.3 Nitrapyrin

The reactive compound of the new nitrification inhibitor N-LOCK^{TM} Nitrogen Stabilizer, listed in Germany since 2015, is 2-Chloro-6-(trichloromethyl)-pyridine, better known as Nitrapyrin. Nitrapyrin is a substituted heterocyclic N compound which is highly soluble in organic solvents (*McCarty and Bremner*, 1989).

It is assumed, that the main mode of AMO inhibition is the Cu-chelating effect of this active compound and is caused by the chloride Cl- (chloride) and CCl_3-group substituted on C atom adjacent to the ring N atom (*McCarty*, 1999). However, *Vannelli and Hooper* (1992) observed a non-competitive as well as an apparently weak mechanism-based inhibition of the AMO by Nitrapyrin. Thus, a strict classification of Nitrapyrin in only one group of inhibitory mechanism is unfeasible. Due to the relative high vapor pressure, an incorporation in 5 up to 10 cm soil depth is necessary to prevent volatilization of this reactive substrate (*Subbarao et al.*, 2006). Furthermore, the release of Nitrapyrin into the environment was highly debatable because of its belonging to the organic chlorine compounds (*Zerulla et al.*, 2001). *Trenkel* (1997) confirmed a different toxicological problem and referred to corrosive and explosive properties of Nitrapyrin. The new formulation of Nitrapyrin as N-LOCK^{TM} should stop the moderate volatility of Nitrapyrin through its capsuled suspension and simultaneously increase the inhibitory effect. N-LOCK^{TM} is suitable for the scope of slurry and digestate application (*The Dow Chemical*

Company, 2012). But Nitrapyrin is limited in its inhibitory efficiency due to a medium soil mobility (lower than NH_4^+) (*Pasda et al.*, 2001) and its tendency to bind to OM in soils (*Keeney*, 1986). *Hendrickson and Keeney* (1978) and *Sahrawat et al.* (1987) reported, that the impact of Nitrapyrin as NI in organic soils (Histosols) is ineffective due to the adsorption by SOM and following a reduced mobility, bioactivity, effectiveness and a prolonged persistence. Regarding soil pH, it was reported, that Nitrapyrin is stable in a range between 2.7 and 11.9 (*Slangen and Kerkhoff*, 1984). It was observed, that Nitrapyrin was most effective in the slightly acidic to neutral pH level, whereby the higher sensibility of nitrifiers to Nitrapyrin in this range is largely caused by the relationship between pH and ecology of target nitrifying organisms (*Hendrickson and Keeney*, 1978; *Slangen and Kerkhoff*, 1984). The temperature and soil moisture content has also an impact on the effectiveness of Nitrapyrin. The hydrolysis of this NI increases with higher soil moisture and soil temperature (*Hendrickson and Keeney*, 1979). Normally, Nitrapyrin decomposes within 30 days under warm soil conditions which usually dominate during maize growth. However, it is very persistent and stable under colder soil conditions with a half-life of 43 up to 77 days at 10 °C, but only 9 up to 16 days at 20 °C (*Slangen and Kerkhoff*, 1984).

Thompson et al. (1987) observed the effect of cattle slurry injection with and without Nitrapyrin during winter and spring regarding N_2O emissions and NO_3^- dynamics. The cumulative N_2O losses through denitrification were strongly reduced by Nitrapyrin addition from 53 kg N ha^{-1} to 23 kg N ha^{-1} in winter and 18 kg N ha^{-1} to 14 kg N ha^{-1} in spring caused by a delayed nitrification. The lower gaseous N losses from Nitrapyrin treatments were also reflected in a higher N recovery of grass herbage in both experiments. Similarly, *Comfort et al.* (1990) reported a significant reduction of N_2O emissions and NO_3^- concentrations in soil after dairy slurry injection through Nitrapyrin addition over 40 days. The highest N_2O fluxes occurred in the first days after injection with a subsequent increase of the N_2/N_2O ratio.

Nitrapyrin has been largely evaluated under different crops and climatic conditions (*Prasad and Power*, 1995; *Rodgers*, 1986; *Slangen and Kerkhoff*, 1984). Through the addition of Nitrapyrin to manure application, *Sutton et al.* (1986) observed an average increase of maize yield by 6 % in a 3-year field study. The study from *McCormick et al.* (1984) did not generate such clear results regarding maize yield through the use of Nitrapyrin together with swine manure injection. Within a 3-year research period, the achieved results were inconsistent. They concluded that the addition of Nitrapyrin had only a beneficial effect on maize yield, if no excessive amount of N was available for plants.

2.5.4.4 1,2,4 Triazole and 3-Methylpyrazole (TZ & MP)

The solution of the two pyrazole derivates TZ & MP, marked under the trade name PIADIN®, contains two active substances (3.00-3.25 % TZ; 1.50-1.65 % MP (*SKW Stickstoffwerke Pisteritz GmbH*, 2007)) inhibiting nitrification separately (*McCarty and Bremner*, 1989). Both active compounds belong to the heterocyclic N compounds with two adjacent N atoms, which were reported to increase the inhibition effect strongly (*McCarty*, 1999). PIADIN® is suitable for liquid organic fertilizers. Up to now, only few studies were conducted using this inhibitor. The reported results regarding N_2O emissions, yield and NUE were controversial, probably due to varying experimental conditions and observation periods (*Barneze et al.*, 2015; *Federolf et al.*, 2016; *Hu et al.*, 2013; *Misselbrook et al.*, 2014; *Spott et al.*, 2015; *Wolf et al.*, 2014).

Wolf et al. (2014) reported a significant reduction of N_2O releases through PIADIN® addition the weeks following digestate incorporation, whereas annual N_2O emissions, maize biomass and grain yield were not affected. *Spott et al.* (2015) detected a significant reduction of nitrification rates and N_2O emissions through adding PIADIN® over 29 days in an incubation experiment after placing slurry depots into soil. *Hu et al.* (2013) even detected a significant N_2O reduction over one entire year which were reduced by 55 % through adding this inhibitor to urea and straw application. On the contrary, in a 54-day field experiment from *Barneze et al.* (2015), the NI-induced N_2O reduction of 6 % in combination with cattle urine application could not statistically be secured. However, *Federolf et al.* (2016) observed significant increase of N removal from maize plants when adding PIADIN® to swine manure injection.

2.5.4.5 Dicyandiamide (DCD)

The active compound DCD was detected as NI in the early 1920s (*Prasad et al.*, 1971). The mainly inhibitory mechanism of DCD is the Cu-chelating effect. Thereby, DCD has a specific bacteriostatic and no bactericidal effect on *Nitrosomonas*, that means that DCD only suppresses biological activity, it neither kills bacteria nor has a general inhibitory effect on biological activity, excluding nitrification (*Amberger*, 1989, 1986). DCD is a water-soluble, non-volatile N stabilizer, which is suitable for cattle manures and animal slurries (*Amberger*, 1989). DCD degradation and efficiency depends on temperature, moisture, texture, pH and OM content of the soil.

A study which included six different soils determined the half-live time of DCD at different soil temperatures (*Kelliher et al.*, 2008). The half-live time of DCD was nearly 120 days at a mean soil temperature of 8 °C, but strongly decreased with rising soil temperatures and achieved a half-live time of approximately 20 days at 20 °C. Similarly, *Vilsmeier* (1980) reported an inhibitory effect of DCD up to 80 days at a soil temperature

of 8 °C and a rapid decrease of its persistence to 20 or 40 days when temperatures increased to 20 °C. *Irigoyen et al.* (2003) confirmed these findings and detected an inhibitory effect of DCD for 3 months at 10 °C but only for one week at 30 °C.

The several-fold higher mobility of DCD compared to NH_4^+ easily promotes leaching out of the root zone and consequently reduces its effectiveness (*McCarty and Bremner*, 1989; *Pasda et al.*, 2001), especially in water-saturated light textured soils (*Ruser and Schulz*, 2015; *Zerulla et al.*, 2001). Generally, the inhibitory effect of DCD decreases with rising soil moisture content (*Hendrickson and Keeney*, 1979). Additionally, *Slangen and Kerkhoff* (1984) reported an increased DCD degradation in sandy loam soils with high SOM content due to the utilization of DCD as N source by heterotrophic microorganisms. *Puttanna et al.* (1999) reported a decreasing inhibition of DCD with rising pH level, where they explained this effect with an increased biological activity of nitrifiers and heterotrophs.

The effectiveness of DCD as NI regarding NO_3^- leaching, N_2O emissions and improved NUE was confirmed by numerous study under different crops and climatic conditions (*Amberger*, 1989; *Di and Cameron*, 2004, 2002).

Vallejo et al. (2005) investigated the effect of pig slurry injection and surface application on N_2O emissions and NO_3^- leaching, where injection was carried out with and without DCD addition. The total N_2O-N loss in percent of applied N was 1.60 % for surface application, 2.95 % for injection and 0.50 % for slurry injection with DCD over a period of 215 days. The reduction of N_2O emission by DCD addition was also reflected in significant lower NO_3^- leaching losses. After a surface application of cattle slurry on grassland, the addition of DCD also reached a N_2O reduction of 60 % in comparison to sole slurry application (*Merino et al.*, 2002).

2.6 References

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3 Effect of cattle slurry application techniques on N_2O and NH_3 emissions from a loamy soil

3.1 Abstract

A full assessment of the effect of organic N-fertilizers on the atmospheric burden is difficult due to the fact that only few studies combined nitrous oxide (N_2O) and ammonia (NH_3) emissions which may vary considerable as a result of different environmental conditions, and due to the lack of full annual N_2O measurements. In this context we determined N_2O fluxes from an unfertilized control (CON), from a treatment with mineral N-fertilizer (MIN), after banded cattle slurry surface application with subsequent incorporation (INC) or after slurry injection (INJ) to silage maize on a Haplic Luvisol in Southwest Germany over two experimental years. NH_3 losses were measured after fertilizer application in the second year and estimated for both years whereby measured and estimated data in the second year were significantly correlated (r^2=0.96). N-fertilization (amount of total N applied + initial N_{min} content) was 210 kg N ha^{-1} in both years. Since the amounts of N-fertilizers were based on total N, the share of inorganic N in the INC and INJ treatments was only half when compared to the MIN treatment. In the first year, NH_3 emissions in the INJ treatment were smaller (15 % of applied ammonium (NH_4^+)-N) than in the INC treatment (23 %). In the second year, NH_3 losses from these two treatments were similar (12 %). The height of the NH_3 emission from the INC treatment depended strongly on the environmental conditions in the time gap between surface application and the incorporation. The mean direct N_2O emission over both experimental years was 2.8, 4.7, 4.4 and 13.8 kg N_2O-N ha^{-1} yr^{-1} for the CON, MIN, INC and INJ treatment, respectively. Across all treatments and years, direct N_2O emission was the major contributor to total greenhouse gas (GHG) emission from silage maize cultivation with an average of 79 %. GHG emissions on field level, including N losses, net CH_4 fluxes, fuel consumption and pre-chain emissions from mineral fertilizer were lowest for INC treatment, followed by MIN treatment and significantly highest for INJ treatment. Consequently, a trail hose application with immediate incorporation was evaluated as the optimal management practice for livestock farmers at our study site.

3.2 Introduction

With a share of 7.9 % N_2O contributes substantially to the annual anthropogenic GHG emissions (*Rogner et al.*, 2007). Furthermore, N_2O is involved in stratospheric ozone depletion (*Ravishankara et al.*, 2009). In Germany, approximately 81 % of the total anthropogenic N_2O emissions in 2015 originated from the agricultural sector (*UBA*, 2017). This large proportion was mainly caused by direct N_2O emissions derived from

fertilized agricultural soils where manure spreading accounted for 12 % of the total national anthropogenic N_2O emission (*UBA*, 2017).

The production of N_2O in cultivated soils is mainly stimulated by the N input through mineral or organic fertilizers which provides the substrates for N_2O production through microbial N conversion processes, like nitrification, denitrification and/or nitrifier-denitrification (*Arp and Stein*, 2003; *Azam et al.*, 2002; *Wrage et al.*, 2001). Nitrification and denitrification are the two main sources of N_2O production, whereas the major part of N_2O emission was attributed to denitrification, especially under conditions with low oxygen (O_2) availability (*E.J. Bateman and Baggs*, 2005). In contrast to mineral fertilization, organic N-fertilizers further stimulate microbial activity through the additional supply of easily available carbon which may result in more anaerobic conditions due to the higher O_2 consumption through enhanced microbial respiration (*Flessa and Beese*, 2000; *Giles et al.*, 2012).

NH_3 mainly known to give rise to eutrophication and acidification of environmentally sensitive areas and water resources (*EEA*, 2013), additionally contributes indirectly to GHG emissions because it induces the formation of N_2O after NH_3 deposition on nearby soils (*Nevison*, 2000). In Germany 5 % of the total annual N_2O emissions were attributed to indirect N_2O emissions due to NH_3 deposition from agricultural soils with organic fertilizers applied as the main source. More than half of the NH_3 emissions in German dairy cattle farming systems occur during the application of slurry (*Rösemann et al.*, 2017).

It has already been shown, that the application technique of organic fertilizers has an immense impact on the magnitude of direct N_2O and NH_3 emissions (*Sommer and Hutchings*, 2001; *Wulf et al.*, 2002a, 2002b) and thus, the optimization of the application technique was identified as an appropriate approach to reduce gaseous N losses and optimize ecological and economical requirements.

Up to 90 % of applied NH_4^+-N can be lost as NH_3 after spreading of animal slurry (*Webb et al.*, 2005). Generally, NH_3 emission after slurry application increases with increasing contact time and exchange area of the slurry with the atmosphere. Both are related to the application technique and/or the incorporation management (*Sommer and Hutchings*, 2001). Furthermore NH_3 emission is influenced by weather conditions and enhanced with rising wind velocity, solar radiation and air temperature (*Sommer et al.*, 2003). Consequently, slurry injection has often been suggested as a promising tool to reduce NH_3 emissions effectively with a lower weather-dependence when compared to surface application with subsequent incorporation (*Mattila*, 2006; *Sommer and Hutchings*, 2001; *Wulf et al.*, 2002a).

Comparing different application techniques, slurry injection significantly increased direct N_2O emission when compared to broadcast application (*Velthof and Mosquera*, 2011; *Wulf et al.*, 2002b), where the soil area directly above the injection slot was the main emitting area (*Dittert et al.*, 2001) due to creation of anaerobic hotspots in the injection slot promoting denitrification (*Flessa and Beese*, 2000).

Unfortunately, most of the direct N_2O emissions reported from the studies mentioned above, are based on non-annual data sets, with N_2O flux measurements exclusively in the time period after fertilization. These data sets are hardly comparable because they covered different measurement periods and they further carry the risk of underestimating direct N_2O emissions mostly in regions with intense freeze/thaw cycling. It was shown that the emissions during freeze/thaw can contribute substantially (≈ 50 %) to the total annual N_2O emission in our experimental region (*Jungkunst et al.*, 2006; *Kaiser and Ruser*, 2000). Thus, annual data sets are a prerequisite for a reliable assessment of practical measures to reduce direct N_2O emission from agricultural soils (*Webb et al.*, 2010). Based on calculated data, it was shown for different cropping systems that N_2O emissions from soils are the largest contributor to total GHG emission on field level (*Camargo et al.*, 2013; *Jacobs et al.*, 2017). However, the authors criticized the high uncertainty and the missing adaption of site-specific N_2O emission factors (EF). Consequently, *Camargo et al.* (2013) and *Jacobs et al.* (2017) arrogated additional research on quantifying N_2O emission from crop production with field measurements. *Pucker et al.* (2013) used an application technique-specific N_2O EF for the calculation of field-related N_2O emissions after digestate fertilization according to *Wulf et al.* (2005, 2002b). They reported a nearly 3-times higher N_2O EF for injection compared to trail hose application. Unfortunately, this EF was based on field measurements covering only 42 days. Consequently, the relevance of increased N_2O emission from cattle slurry injection on the overall GHG balance on field-scale remained uncertain.

Beside the objective to determine an application technique which improves the internal N cycle and avoids extra costs for N-fertilizers (*Dawson et al.*, 2008), the reduction of GHG emissions, including gaseous N losses from agricultural sector is a major global challenge and a declared national and international political objective to protect the earth's climate and the environment (*Flessa et al.*, 2002; *UBA*, 2017). Also the National German Greenhouse Gas Inventory (*UBA*, 2017) reported a total uncertainty of 38.1 % for emissions from animal husbandry, agricultural soils and digestion of energy crops, which was mainly caused by the uncertainty for the N_2O emissions from agricultural soils. Moreover, in order to identify the best management practice to reduce gaseous N losses from agricultural soils, *Webb et al.* (2010) also arrogated field studies which simultaneously determine NH_3 volatilization and N_2O emission.

Therefore, in the present study annual N_2O emissions were measured in two experimental years whereas NH_3 emissions were measured or estimated after fertilization to verify the following hypotheses: (1) cattle slurry injection increases N_2O emission when compared to surface application with subsequent incorporation whereas the NH_3 emission decreases; (2) winter emissions contribute considerably to the annual N_2O emission due to high N_2O fluxes during freeze/thaw cycles, (3) direct and indirect N_2O emissions contribute substantially to the total GHG emission on field-scale, and (4) slurry injection increases the GHG emission when compared to broadcast application with subsequent incorporation.

3.3 Material and methods

3.3.1 Experimental site, design and crop management

The study was conducted at the research station "Heidfeldhof" (48°43'0.30'' N; 9°11'30.64'' E; 404 m a.s.l.), which belongs to the University of Hohenheim. The long-term mean precipitation is 691 mm yr^{-1} with an annual mean air temperature of 10.1 °C (*Agrarmeteorologie Baden-Württemberg*, 2017). Soil type was a Haplic Luvisol. Soil texture of the stone-free soil was composed of 2 % sand, 74 % silt, and 24 % clay, a typical texture for the periglacial loess derived soils in this region. The initial pH in the A_p horizon (0-30 cm depth) was 7.0 (10^{-2} *M* $CaCl_2$). C_{org} and N_t content of the top soil was 0.72 % and 0.10 %. Measurements were carried out on two adjacent fields between 30 April 2015 and 19 April 2016 in the first experimental year, and between 28 April 2016 and 3 May 2017 in the second experimental year.

The experimental design on both fields was a fully randomized block design with four replicates and a plot size of 10 m x 3 m. The treatments were (i) an unfertilized control (CON), (ii) broadcast, surface applied mineral fertilization with calcium ammonium nitrate (MIN), (iii) cattle slurry application on the soil surface with a trail hose and subsequent incorporation with a circular harrow after 2 h (15 cm depth) (INC), and (iv) cattle slurry injection with an injecting cultivator (15 cm depth, 5 cm share width) (INJ). The space between the injection tines or trail hoses was 75 cm.

To quantify the effect of different time periods between surface application and incorporation of the slurry in the INC treatment, a further treatment for NH_3 measurements was integrated into the experimental design after fertilization in the second experimental year. For this additional treatment, the slurry was also applied with a trailing hose, but not incorporated. In this way, it was possible to calculate the putative NH_3 losses, when the slurry was incorporated after 3, 4, 5 or 6 hours (h) or remained on the soil surface (no incorporation).

Maize (*Zea mays* L., variety "Amadeo") was sown in both years (12,700 plants ha^{-1}) with a sowing depth of 5 cm and 5 cm next to the injection slot to guarantee an optimum distance for nutrient distribution between the seeds and the injection slot (*Bittman et al.*, 2012).

In the first experimental year, maize was sown on 13 May and harvested on 26 August as silage maize. In the second experimental year, maize was cultivated on 4 May and chopped on 13 September. After harvest, the maize stubbles were mulched and subsequently incorporated with a cultivator. During the remaining observation period, the field lay fallow without any further soil cultivation activity and crop management practice.

The maximum amount of organic N-fertilizer permitted by the German legislation on N-fertilization (*DüV*, 2006) was 170 kg N ha^{-1} yr^{-1}. Due to the relatively low total N content of the slurry in the first, it became clear that it wouldn't be possible to properly inject the whole corresponding slurry volume (approximately 56 m^3 ha^{-1}) with one application. We therefore decided to split slurry application in two doses (Table 3.1).

Table 3.1: Characteristics and amounts of the applied cattle slurry used for organically fertilized treatments (INC, INJ) and the initial N_{min} soil contents (0-60 cm, extracted with $CaCl_2$).

year	date	cattle slurry applied amount / m^3	total N / kg m^{-3}	total NH_4^+-N / kg m^{-3}	DM / %	pH	initial N_{min} / kg ha^{-1}	fertilization / kg N ha^{-1}
2015	1st	44.0	3.1	1.3	8.5	6.6	44	136
	2nd	12.0	2.8	1.4	7.2	6.6		33
2016	1st	50.5	3.7	2.2	n.d.	7.3	21	187
	2nd	0.0	-	-	-	-		

The first dose (140 kg N ha^{-1}) was applied on 10 May, the second dose (30 kg N ha^{-1}) was applied on 18 June. The second dose of the two treatments with cattle slurry (INC and INJ) was only surface-applied with a trail hose because preliminary injection tests showed that the growing maize plants were strongly damaged due to agglomeration of the loamy soil. In the second year, the higher N content of the slurry allowed for a single application on 3 May.

N demand for our maize variety was calculated according to the *DüV* (2006); it amounted to 210 kg N ha^{-1}. Due to the lower initial N_{min} contents in the second year, we applied 190 kg total N ha^{-1}. Consequently, the sum of initial N_{min} and N applied was the same in both experimental years.

The inorganic N amounts applied in the MIN treatment were twice as high as in those of organic fertilized treatments (INC and INJ).

To evaluate the N availability of slurry N for maize in comparison to the MIN treatment, mineral-fertilizer-N equivalents (MFE) were calculated for INC and INJ treatment (Equation 3.1).

Equation 3.1

$$MFE = \frac{(N\ removal_{ORG} - N\ removal_{CON})}{(N\ removal_{MIN} - N\ removal_{CON})}\ x\ 100$$

with MFE: mineral-fertilizer-N equivalents [%], N removal in the particular organic fertilized treatment (N $removal_{ORG}$), in unfertilized control (N $removal_{CON}$) and in mineral fertilized treatment (N $removal_{MIN}$) [kg N ha^{-1}].

3.3.2 Trace gas sampling and flux calculation

During the whole observation period, N_2O and CO_2 trace gas fluxes were measured weekly complemented by additional event based samplings using the closed chamber method (*Hutchinson and Mosier*, 1981). The chambers for the injection treatment were placed area-representatively (7 % injection slot, 93 % next to the injection slot), close to the maize row (*Gassner*, 2017).The chambers of the other treatments were placed in the same way. Chamber design and gas sampling procedure of the trace gases was described in detail by *Pfab et al.* (2011). N_2O and CO_2 concentrations in the samples were determined gas chromatographically (GC 450 Greenhouse Gas Analyzer, Bruker Daltonic, Bremen, Germany). Separation of the trace gases was carried out using a Hayesep D column (80-10 mesh) and an oven temperature of 80 °C. N_2O and CO_2 concentrations were analyzed with a ^{63}Ni electron capture detector (ECD). Chromatograms were integrated using Bruker Compass CDS™ 2012 software.

Trace gas flux rates were calculated using the package "gasfluxes" (*Fuss and Asger*, 2014) for R software (*R Core Team*, 2016), which selects the most suitable model for calculating trace gas fluxes, including numerous factors, which are outlined in depth by *Ruser et al.* (2017). For the calculation of annual N_2O emission, N_2O fluxes from each treatment and experimental year were cumulated, assuming constant flux rates between two gas samplings. Methane (CH_4) fluxes were also detected and reported in Chapter 5.

The N_2O EF related to total N applied was calculated as described by *Velthof and Mosquera* (2011) (Equation 3.2).

Equation 3.2

$$EF = \frac{(N_2O - N\ emission_{fertilized\ treatment} - N_2O - N\ emission_{CON})}{total\ N_{fert}}\ x\ 100$$

with EF: emission factor of total N applied [%]; N_2O-N emission is the mean cumulative direct N_2O-N emission of the particular experimental year either from the treatments fertilized ($emission_{fertilized\ treatment}$) or from the unfertilized control ($emission_{CON}$) [kg

N_2O-N ha^{-1} yr^{-1}], and total N_{fert} is the amount of total N-fertilizer applied as mineral fertilizer or cattle slurry [kg N ha^{-1} yr^{-1}].

3.3.3 NH_3 measurements and calculations

NH_3 volatilization was measured after fertilization in the second year in the treatments INC, INJ, and trail hose application without incorporation using the open dynamic chamber Dräger-tube method (*Pacholski et al.*, 2006). NH_3 emissions from CON treatment were additionally measured to determine background emissions. The measurements were conducted as long as the NH_3 fluxes were higher than in the unfertilized control treatment (6 days after fertilizer application, 4 or 5 times daily).

Table 3.2: Data used as framework conditions for the calculation of NH_3 losses as described by *Horlacher and Marschner* (1990).

	Application date		
	1st 2015	2nd 2015	1st 2016
infiltration	medium	medium	medium
air temperature level, application / °C	19.7	14.1	12.2
air temperature level, precipitation / °C	17.9	14.1	12.2
precipitation level / mm	4.0	11.7	3.3
hours between application and precipitation / h	78.0	1.0	10.0

Since we did not measure NH_3 losses after the first and second slurry application in the first experimental year, we calculated NH_3 emission for injection and all trailing hose applications with the framework suggested by *Horlacher and Marschner* (1990). However, NH_3 emission after slurry injection were not covered by the framework of *Horlacher and Marschner* (1990). As suggested by *Rösemann et al.* (2017), we assumed that the NH_3 emission from the injection treatment was similar to the emission from the treatment with trailing hose application and incorporation within 1 h. Table 3.2 shows the environmental conditions used for these calculations.

3.3.4 Calculation of CO_2 equivalents

To assess the impact of N-fertilization and slurry application technique on total GHG emission, direct N_2O emission, indirect N_2O emission (attributed to NH_3 volatilization and nitrate (NO_3^-) leaching), emission from fuel consumption as well as pre-chain emission from calcium ammonium nitrate (CAN) production and transport were converted to CO_2 equivalents. Therefore we used the default GWP of 298 g g^{-1} CO_2 for N_2O (*IPCC*, 2007) and of 23 g g^{-1} CO_2 for CH_4 (*European Commission*, 2010).

To quantify the percentage of NH_3 emission re-emitted as N_2O-N, we calculated an N_2O EF based on the results of the MIN treatment (Equation 3.2).

For the calculation of indirect N_2O emission via NO_3^- leaching we used equation 11.10 from *IPCC* (2006) guidelines. Thereby, data from our field experiment were used for calculation of amount and N content of aboveground harvest residues (stubble, leaves), assuming that 10 % of silage maize harvested remained in the field (*Jacobs et al.*, 2017).

For belowground harvest residues the *IPCC* (2006) default values for maize were used. Indirect emission as a result of soil N-mineralization and C-stock changes were not considered.

Diesel consumption for tillage, sowing, harvest and the different fertilizer application techniques was calculated using an online-based calculator of the German Association for Technology and Structures in Agriculture (*KTBL*, 2009). For the calculation of site-specific fuel consumption, the distance between farm and field was set to 1000 m, field size was defined with 1 ha and middle soil resistance was assumed. The default value of 74.1 Mg CO_2 Mg^{-1} was exploited for calculation of CO_2 equivalents from diesel fuel, including emissions during production, transport and burning (*Juhrich*, 2016).

For the mineral fertilizer CAN, the CO_2 equivalents from production and field-related NH_3 emissions from applied CAN were taken from *Hillier et al.* (2012), who assumed 3.60 Mg CO_2 Mg^{-1} N. NH_3 losses after CAN application were calculated with equation 11.11 from *IPCC* (2006) guidelines. For the transport of CAN used in Western Europe, the default value of 0.1 Mg CO_2 Mg^{-1} N was assumed (*Brentrup and Pallière*, 2008). Emissions from supply of cattle slurry (animals, housing and storage) were excluded from calculation of field-related GHG release because of no pre-chain emissions by definition (*Olesen et al.*, 2006; *UBA*, 2017).

3.3.5 Weather data, soil sampling and laboratory analyses

Precipitation and air temperature data were provided by the University's meteorological station, which is located approximately 500 m from the experimental site. Additionally, soil temperature in each of the four replicated blocks was recorded in 5, 10, 15 cm soil depth (Logtag, TRIX-8, CIK solutions, Karlsruhe, Germany).

Simultaneously to gas sampling, soil samples (0-30 cm) were taken to determine N_{min} and soil moisture. Except for the INJ treatment, three samples were taken randomly with an auger (inner diameter 2 cm) out of each plot. We then pooled these soil samples from the four replicated plots of each treatment and stored them frozen, before analyses. For the INJ treatment, samples were taken directly over the injection slot and in the area between the slots, separately.

Additionally, soil samples were taken before sowing from each plot separately (0-30 cm, 30-60 cm, and 60-90 cm) to identify the initial N_{min} contents.

To determine mineral N content, 20 g of fresh soil was extracted with 80 mL of a 0.5 *M* K_2SO_4 solution. NO_3^--N and NH_4^+-N concentrations in the samples were measured using flow injection analyses (3 QUAAtro, SEAL Analytical, UK).

A further aliquot of each soil sample was dried at 105 °C for 24 h to determine soil moisture gravimetrically.

3.3.6 Plant analyses

At harvest, the aboveground biomass of the maize plants from two central maize rows was chopped. The maize chopper determined the fresh matter (FM) yield per plot. An aliquot of chopped maize was dried at 60 °C for 4 d and used to calculate dry matter (DM) yield and to determine C and N concentrations (Elementar analyzer, vario MAX CN, Elementar Analysensysteme, Hanau, Germany). The information from plant analyses was used to calculate FM and DM yields as well as the N removals from the field.

3.3.7 Statistical analyses

Statistical analyses were carried out using the Statistical Software package SigmaStat 3.5. Data were tested for variance homogeneity. Normal distribution of residuals was tested with the Shapiro Wilk Test. The dataset from total and yield-related CO_2 equivalents from the first experimental year did not follow a normal distribution, thus a Kruskal-Wallis One Way ANOVA on Ranks was chosen for statistical analyses. The N_2O fluxes from the first experimental year were log_{10}-transformed to realize variance homogeneity. Remaining data showed variance homogeneity without any transformation. A two factorial ANOVA was run with the factors block and treatment, to test block effects in each experimental year.

Owing to differences between the two experimental years (*e.g.* climatic conditions in the period after fertilization, slurry characteristics, abundance of fertilization, change of experimental site) in terms of yield, N removal and cumulative N_2O emissions, a one factorial ANOVA was performed separately for each experimental year to detect differences between the treatments within the particular observation periods. Significant differences were determined using a pairwise multiple comparison procedure (*Student-Newman-Keuls, $p<0.05$, $n=4$*). The data are presented as arithmetic means with standard errors.

3.4 Results

3.4.1 Meteorological conditions

In both experimental years, the mean annual air temperature was quite similar to the long-term annual mean temperature. During the winter season of the first year, the soil temperature below 0 °C was too short a period to freeze soil effectively (Figure 3.1f).

This was in contrast to the second winter season where soil temperature in 5, 10 and 20 cm depth was below 0 °C for 13, 11 and 8 days, respectively, in mid/end January 2017 (Figure 3.1f).

However, the first experimental year was an unusually dry year with approximately 150 mm less precipitation than the long-term annual mean. Especially during the period after

fertilization, in May up to July, the precipitation was 33 % less than expected. In this period, only one heavy rainfall event of approximately 30 mm occurred on 8 June.

Precipitation in the second experimental year was around 50 mm lower than the long-term annual mean. Immediately after fertilization, precipitation was only 11 % below the corresponding long-term mean, indicating that conditions after fertilizer application were representative for our study site. Moreover, intense rainfalls occurred more frequently in the period after fertilization. Heavy rainfall of 49 mm d^{-1} occurred within 3 days before gas sampling on 31 May. Before trace gas sampling on 9 June, 52 mm precipitation were measured within 2 days and further 30 mm precipitation occurred until the following gas sampling on 14 June.

During the first fertilization measure in 2015, air temperature ranged between 15-20 °C, mean gravimetrical soil moisture was 20 % and no precipitation occurred within 3 days after application. The air temperature during the second slurry application in 2015 varied between 10-15 °C, gravimetrical soil moisture amounted to 25 % and it rained immediately after application.

During the slurry application in 2016, the mean air temperature was 12.2 °C and the mean gravimetrical soil moisture amounted to 23 %. The mean daily air temperature during the NH_3 measurements was 9.9 °C and 3.3 mm precipitation occurred 10 h after application. These data were used as framework conditions for the calculation of the NH_3 losses (Table 3.2).

3.4.2 Temporal pattern of the N_2O flux rates and environmental conditions

The N_2O flux rates showed a high temporal variability during the whole observation period (Figure 3.1a and Figure 3.2a). High N_2O fluxes were measured after N-fertilization with simultaneously high soil moisture contents. Very high N_2O pulses occurred especially after rewetting of dry soil in summer. In both years, highest mean flux rates were measured in the slurry injection treatment (INJ) with 6720 µg N_2O-N m^{-2} h^{-1} in the first and 2422 µg N_2O-N m^{-2} h^{-1} in the second year. In the course of the vegetation periods, the N_{min} soil contents (Figure 3.1b, c and Figure 3.2b, c) and the gravimetric soil water content (GSW) (Figure 3.1e and Figure 3.2e) decreased noticeably with the progressive growth of the maize plants. Harvest and incorporation of residues increased the N_2O fluxes just marginally in both years. From the middle of vegetation periods and subsequent black fallows, the N_2O flux rates remained mainly low. Only in January 2017, increased N_2O fluxes were measured for the slurry fertilized treatments after a freeze/thaw event. Unfortunately, it was not possible to take soil samples during this freeze/thaw cycle period due to the soil conditions and therefore we could not include these increased flux rates into our analyses of correlations between soil NO_3^- contents and N_2O emission rates.

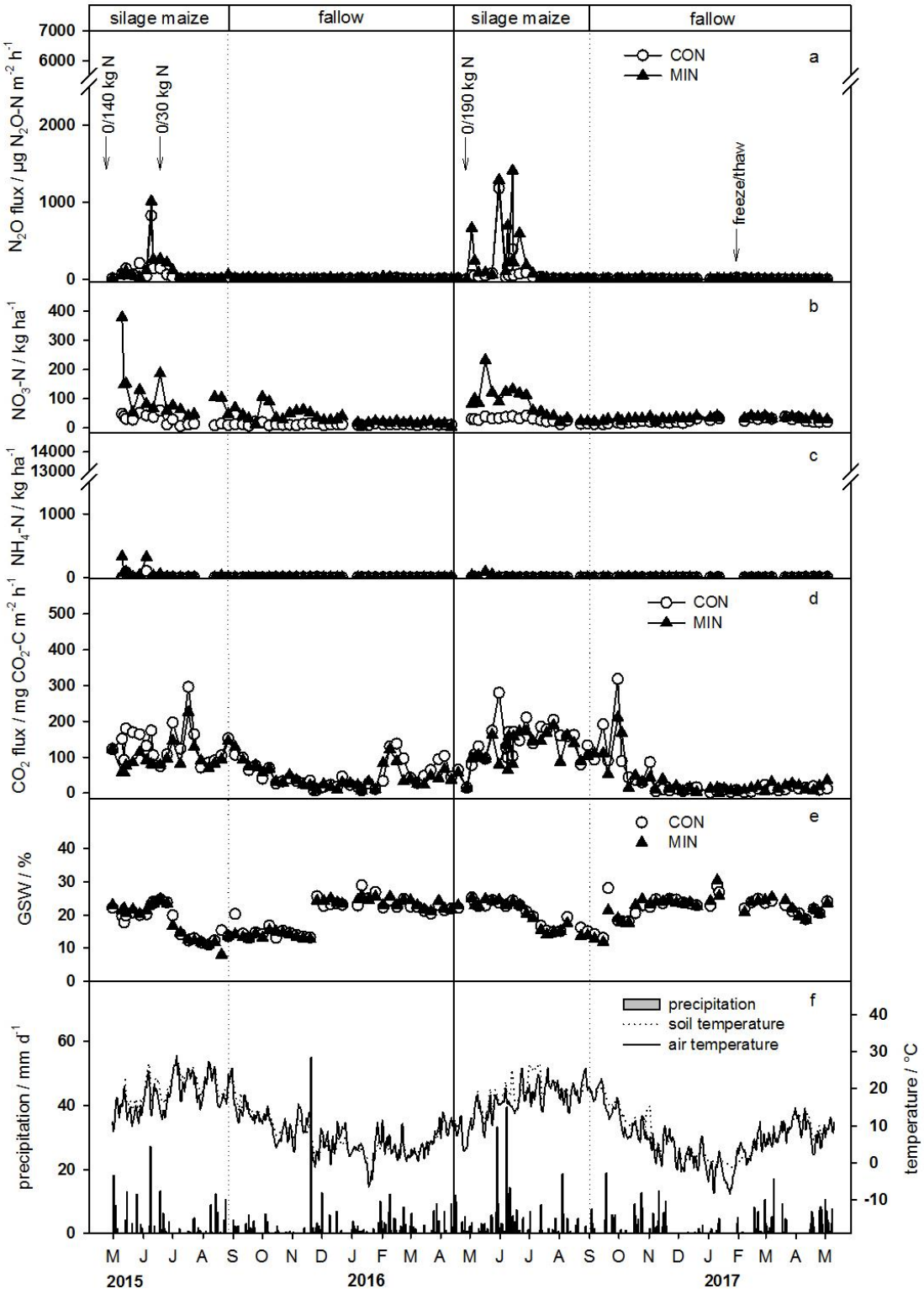

Figure 3.1: Mean N_2O flux rates (*n=4*) (a), NO_3^--N contents (0-30 cm) (b), NH_4^+-N contents (0-30 cm) (c), mean CO_2 flux rates (*n=4*) (d) and gravimetric soil water content (0-30 cm) (e) in the unfertilized control treatment (CON) and the mineral N-fertilization treatment (MIN) over the two experimental years. Arrows indicate fertilization measures in the MIN treatment. Mean air temperature (solid line), soil temperature at 10 cm depth (dotted line), and daily precipitation (black bars) (f). Error bars were omitted due to clarity.

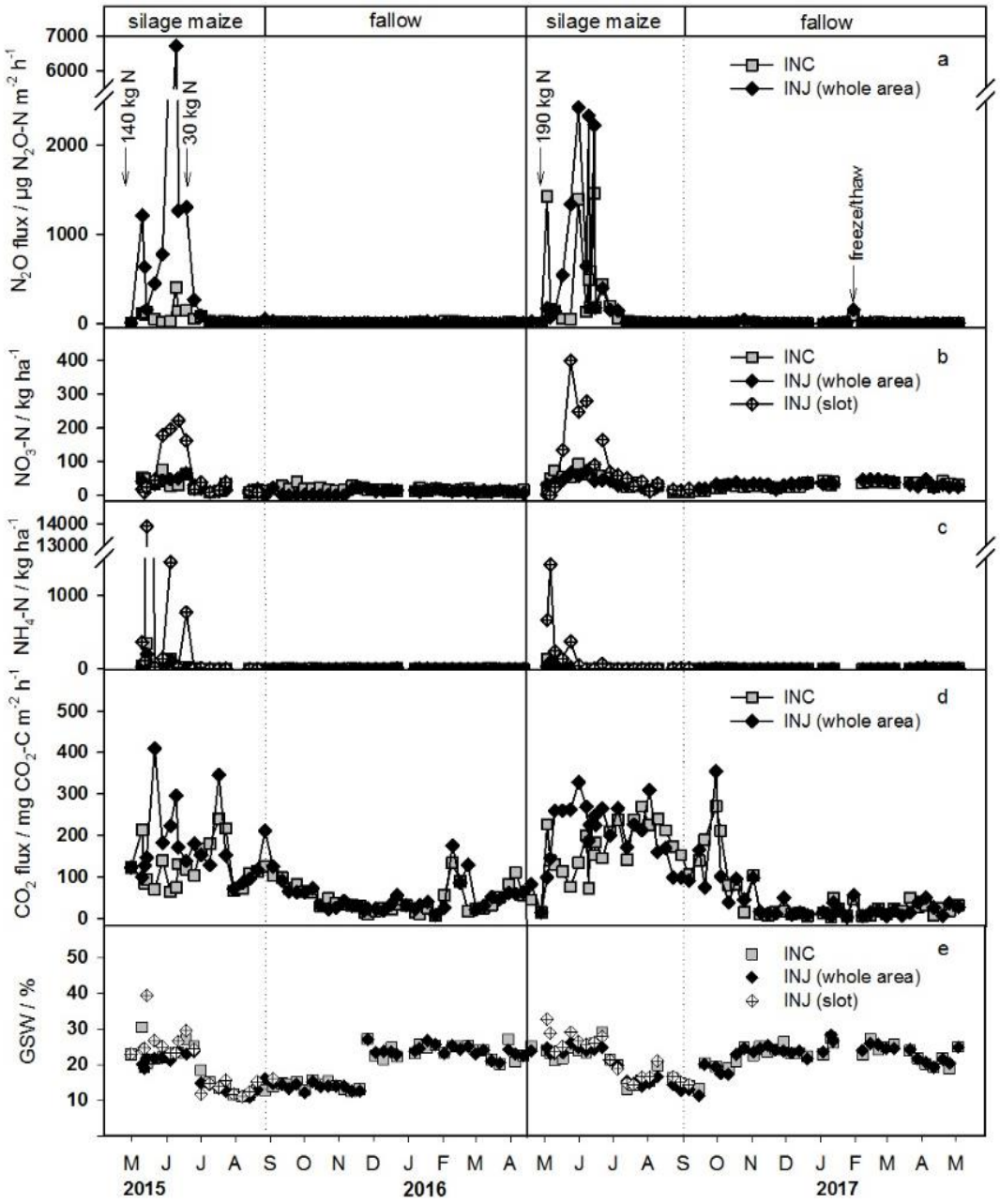

Figure 3.2: Mean N_2O flux rates (*n=4*) (a), NO_3^--N contents (0-30 cm) (b), NH_4^+-N contents (0-30 cm) (c), mean CO_2 flux rates (*n=4*) (d) and gravimetric soil water content (0-30 cm) (e) in the cattle slurry incorporation treatment (INC) and the cattle slurry injection treatment (INJ (whole area)) over two experimental years. Additionally, the NO_3^--N and NH_4^+-N content and gravimetric soil water content of the injection slot area (INJ (slot)). Arrows indicate fertilization measures. Error bars were omitted due to clarity.

Except for the INJ treatment, the N_2O fluxes were generally lower in the dryer first year (Figure 3.1f) with a lower mean soil moisture (Figure 3.1e and Figure 3.2e) than in the second year. Increased N_2O fluxes with rising NO_3^- availability after fertilization and heavy rainfall events were observed often in the fertilized treatments and appeared in a higher frequency in the second year.

The CO_2 flux rates can be interpreted as an indicator for microbial activity. For both experimental years, we found a significant correlation between the CO_2 fluxes and soil temperature in 10 cm depth (r^2=0.40 in the first and r^2=0.50 in the second year, Pearson Product Moment Correlation, $p<0.05$). N_2O fluxes were also correlated significantly with soil respiration (r^2=0.14 in the first and r^2=0.10 in the second year). Although this weak but statistically significant relationship, mean CO_2 fluxes were the best predictor for the variability of N_2O fluxes in the first year, followed by GSW, which could explain the fluctuation by 12 % and 17 %, respectively (Stepwise forward regression). In the second year, the best predictors for the variability of N_2O fluxes were the NO_3^- contents in the topsoil, followed by soil respiration and moisture (14, 22 and 24 % of the variability of N_2O flux rates could be explained, respectively).

3.4.3 Annual N_2O emissions

The cumulative N_2O emissions varied between 2.1 kg N_2O-N ha^{-1} yr^{-1} (INC, first year) and 16.2 kg N_2O-N ha^{-1} yr^{-1} (INJ, first year) (Figure 3.3). In both experimental years, the high N_2O fluxes after slurry injection resulted in significantly higher N_2O emission

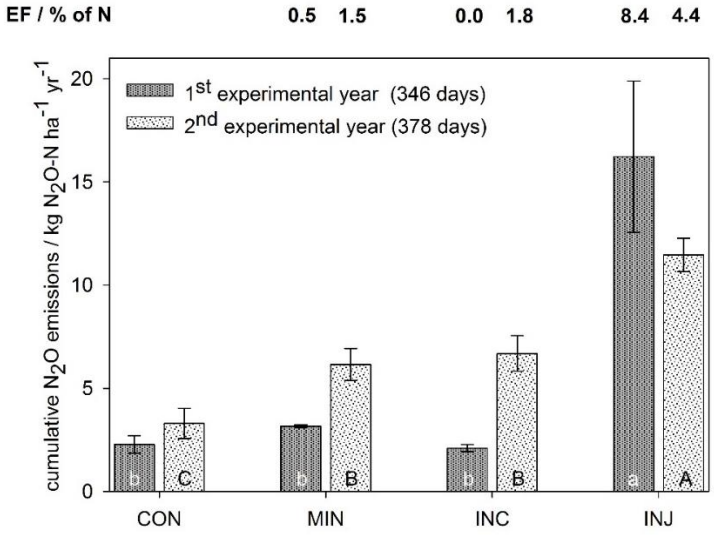

Figure 3.3: Mean cumulative direct N_2O emissions ($n=4$ ± standard errors) as affected by application technique and N-fertilizer (mineral or organic) over 346 days in the first (lower-case letter) and over 378 days in the second (upper-case letter) experimental year. The EF (emission factor) value indicates mean direct N_2O emission related to total N applied of each treatment and year. Different letters indicate statistically significant differences between groups within the same year (*Student-Newman-Keuls test*, $p<0.05$).

compared to all other treatments. In the first year, they were five times higher than in the MIN treatment and about seven times higher than in the CON or the INC treatment. In the second year, the emission from the INJ treatment was approximately 3.5 times higher than in the CON treatment and nearly twice as high as in the MIN and INC treatment.

In the first experimental year, we did not find statistically significant differences between the CON, MIN and INC treatment (Figure 3.3). In contrast, the N_2O emission from the MIN and INC treatment exceeded the emission from the unfertilized control (CON) significantly in the second experimental year.

3.4.4 NH_3 emissions after slurry application

From fertilization in 2016 onwards, measured NH_3 emission data were available. The calculated NH_3 losses correlated significantly with the measured NH_3 losses over all treatments (r^2=0.96, Pearson Product Moment Correlation, $p<0.001$).

The calculated NH_3 volatilization among the treatments with slurry application in the first year was lowest in the INJ treatment (8.6 kg NH_3-N ha^{-1}). Highest NH_3 loss was calculated for the broadcast application without incorporation (42.9 kg NH_3-N ha^{-1}).

Table 3.3: Effect of different application techniques and of varying time frames between surface application and incorporation of the cattle slurry on relative and total NH_3 losses in 2015 and 2016. Cumulative NH_3 losses were calculated based on the underlined data. Different superscript letters indicate statistically significant differences between treatments within the same year (*Student-Newman-Keuls test, $p<0.05$, $n=4$*).

year	date	application technique[a]	estimated NH_3 losses[b] / % of applied NH_4^+-N	measured NH_3 losses / % of applied NH_4^+-N	cumulative NH_3 losses / kg NH_3-N ha^{-1}
2015	1st	injection	<u>15.0</u>	n.d.	8.6
		incorporation after 2 h	<u>22.5</u>	n.d.	12.9
		trailing hose	<u>75.0</u>	n.d.	42.9
	2nd	trailing hose	<u>8.3</u>	<u>n.d.</u>	1.4
2016	1st	injection	8.3	<u>11.6</u>^{d}	12.9^{d}
		incorporation after 2 h	13.8	<u>11.6</u>^{d}	12.8^{d}
		incorporation after 3 h	16.5	<u>15.0</u>^{c}	16.7^{c}
		incorporation after 4 h	19.3	<u>17.4</u>^{b}	19.4^{b}
		incorporation after 5 h	21.3	<u>18.9</u>^{b}	21.1^{b}
		incorporation after 6 h	23.4	<u>19.3</u>^{b}	21.5^{b}
		trailing hose	40.2	<u>45.7</u>^{a}	50.7^{a}

[a] Slurry was applied by trailing hose before incorporation with a circular harrow. Injection was carried out using a slurry cultivator.

[b] Framework for estimation from *Horlacher and Marschner* (1990) was used to calculate NH_3 losses.

In 2016, trail hose application and harrowing after 2 h (INC) showed the same low NH_3 losses as the INJ treatment. Incorporation after 3 h already exceeded NH_3 emissions from prior incorporation significantly (Table 3.3). According to the last German fertilizer ordinance (*DüV*, 2017), surface-applied slurry has to be incorporated into bare soil

within 4 h after application. Utilizing the established maximum time frame of 4 h, significantly increased NH_3 losses occurred (by 6.6 kg NH_3-N ha^{-1}) when compared to an earlier incorporation. There were no differences in NH_3 emissions between the treatments with incorporation after 4, 5 or 6 h. Generally, NH_3 emissions were highest immediately after application of the cattle slurry (Table 3.3). When the slurry was just surface-applied by trail hose and remained there without incorporation, NH_3 losses accounted for almost 50 % of applied NH_4^+-N.

3.4.5 Maize yield and N removal

In 2015, fresh matter (FM) yields ranged between 38.5 Mg ha^{-1} FM in the unfertilized CON treatment and 50.1 Mg ha^{-1} FM in the MIN treatment (Table 3.4). With 180 kg N ha^{-1} the MIN treatment significantly exceeded all other treatments regarding N removal in that year. Slurry injection (INJ) tended to have higher FM yield and N removal compared to INC treatment. The INC treatment had the lowest mean FM yield and N removal among the fertilized treatments (Table 3.4) and it did not differ from the unfertilized control.

Table 3.4: Mean fresh matter (FM) and dry matter (DM) yields and N removal of silage maize with standard errors (SE) as affected by treatment (unfertilized control (CON), mineral fertilization (MIN), cattle slurry surface application with subsequent incorporation (INC), and cattle slurry injection (INJ) and experimental year. Different superscript letters indicate statistically significant differences between treatments within the same year and column (*Student-Newman-Keuls test, $p<0.05$, $n=4$*).

Treatment	FM yield / Mg ha^{-1}	SE	DM yield / Mg ha^{-1}	SE	N removal / kg ha^{-1}	SE
2015						
CON	38.5^{c}	1.0	12.2^{b}	0.4	116^{c}	3.7
MIN	50.1^{a}	1.7	15.1^{a}	0.5	180^{a}	7.8
INC	42.3^{bc}	0.9	12.4^{b}	0.3	132^{bc}	10.3
INJ	45.7^{ab}	2.1	14.1^{a}	0.6	147^{b}	8.1
2016						
CON	27.8^{b}	2.8	10.8^{c}	1.2	78^{c}	6.7
MIN	40.8^{a}	0.7	18.2^{a}	0.3	182^{a}	3.9
INC	33.9^{a}	0.7	14.6^{b}	0.3	120^{b}	2.4
INJ	36.4^{a}	2.6	15.4^{ab}	1.4	140^{b}	11.0

In 2016, the lowest FM was recorded with 27.8 Mg ha^{-1} in the CON treatment, again mineral fertilization obtained the highest yield (40.8 Mg ha^{-1} FM). Yield response and N removal in the second experimental year showed the same trends as in the first year, however the differences between the treatments were much clearer (Table 3.4). FM as well as N removal was significantly lowest in the unfertilized CON. Within the fertilized treatments, INC tended to exhibit lowest yield and N removal but it did not differ from the INJ treatment. The MIN fertilization tended to have the highest yield, but only N removal was significantly higher than the remaining fertilized treatments.

The MFE for the slurry in the INJ treatment was 48 % and 60 % in 2015 and 2016, respectively. The corresponding values for the INC treatment were 25 % in the first and 40 % in the second year.

Over all treatments, FM and dry matter (DM) were significantly correlated with each other. DM yields explained 93 % and 97 % of the variability of the FM yields in 2015 and 2016, respectively (Pearson Product Moment Correlation, $p<0.05$). Consequently, additional discussion of DM yields was ignored due to the same treatment-specific reactions in both years.

3.4.6 Effect of fertilizer type and application technique on GHG emission

Area-related GHG emissions during the production of silage maize showed in both years the same order: INJ > MIN > INC > CON (Table 3.5). It ranged between 1279 kg CO_2 ha^{-1} yr^{-1} (CON, first year) and 8324 kg CO_2 ha^{-1} yr^{-1} (INJ, first year).

Due to the disuse of N-fertilizer, only direct N_2O emission, fuel consumption and NO_3^- leaching losses (provoked by N input through harvest residues) contributed to GHG emission from the unfertilized CON treatment (Table 3.5). Direct N_2O emission accounted for more than 85 % of the total GHG emission (mean of both years) in this treatment. In the MIN treatment, CAN fertilizer production and transport contributed substantially to total GHG emission (25 % in the first year and 17 % in the second year).

In both years, direct N_2O emissions accounted for the highest share to total GHG emission in the organically fertilized treatments (mean 83 % of total GHG emissions), followed by fuel consumption (8 %), NO_3^- leaching (7 %), NH_3 emission (2 %) and CH_4 emission (<1 %) (Table 3.5).

Yield-related GHG emission ranged between 105 kg CO_2 Mg^{-1} DM (CON, first year) and 583 kg CO_2 Mg^{-1} DM (INJ, first year) (Table 3.5). In both years, yield-related emission from the INJ treatment was significantly higher than in all other treatments whereas the emission from the INC treatment did not differ statistically from emission from the unfertilized CON treatment.

Table 3.5: Mean greenhouse gas (GHG) emissions as affected by treatment (unfertilized control (CON), mineral fertilization (MIN), cattle slurry surface application with subsequent incorporation (INC), and cattle slurry injection (INJ)) and experimental year as CO_2 equivalents (CO_2 eq.) calculated from field-related direct N_2O and NH_3 emissions, NO_3^- leaching, CH_4 emission, CAN fertilizer (including emissions from production and transport) and fuel consumption. Different superscript letters indicate statistically significant differences between treatments within the same year and column (*Student-Newman-Keuls test* for dataset from first (*ANOVA on Ranks*; *p=0.005, n=4*) and second year (*ANOVA*; *p<0.05, n=4*)). Data from CH_4 emissions are taken from Chapter 5.

year	treatment	percentage of the total CO_2 eq.						total CO_2 eq.	total CO_2 eq. per yield unit
		N_2O emission	NH_3 emission	NO_3^- leaching	CH_4 emission	CAN fertilizer	fuel consumption	[kg ha^{-1} yr^{-1}]	[kg CO_2 Mg^{-1} DM]
1st	CON	83.4	0.000	2.5	-1.5	0.0	15.5	1279.6^{c}	105.3^{c}
	MIN	59.3	0.003	8.9	-1.4	25.0	8.1	2494.8^{b}	166.3^{b}
	INC	65.2	4.320	14.2	-1.8	0.0	18.1	1505.9^{c}	121.7^{c}
	INJ	91.3	0.546	2.6	2.1	0.0	3.5	8324.0^{a}	582.5^{a}
2nd	CON	88.2	0.000	1.5	-0.5	0.0	10.9	1750.9^{C}	163.4^{B}
	MIN	71.7	0.002	6.2	-0.1	17.4	4.8	4019.1^{B}	222.5^{B}
	INC	85.6	1.602	6.5	-0.6	0.0	7.0	3656.6^{B}	250.0^{B}
	INJ	90.0	0.986	4.0	0.3	0.0	4.7	5963.3^{A}	392.5^{A}

3.5 Discussion

3.5.1 Effect of application technique, fertilizer type and environmental factors on N_2O flux rates

The high temporal variability of N_2O fluxes observed was often reported for arable soils with similar silty texture in South Germany (*Pfab et al.*, 2012; *Ruser et al.*, 2001). Highest N_2O fluxes were measured in periods with increased N_{min} availability in conjunction with high soil moisture and mainly explained with stimulated denitrification after mineral N-fertilizer application (*Pfab et al.*, 2011; *Zebarth et al.*, 2008) as well as after organic N amendment (*Flessa and Beese*, 2000; *Wulf et al.*, 2002b). In both years, N_2O flux rates were positively correlated with soil moisture and NO_3^- contents of the top soil. Consequently, N_2O release decreased approximately 7 to 8 weeks after N-fertilization in both years with decreasing N_{min} contents and decreasing soil moisture. In agreement with *Comfort et al.* (1990), we assume that the high N_2O fluxes of slurry fertilized treatments one day after amendment were caused by the higher soil moisture through the water addition by slurry application which enhanced reduction of soil derived NO_3^- in both experimental years. In due course of time, it is supposed that the nitrified slurry NH_4^+ was the main substrate for N_2O release (*Dittert et al.*, 2001). This assumption is reflected in the high N_2O fluxes with increased soil NO_3^- contents under denitrifying conditions, particularly in the INJ treatment where easily available C and mineral N were concentrated in the injection spot.

Thus, intense oxygen consumption through mineralization of slurry C might have resulted in the creation of anaerobic microsites with intense denitrification activity (*Flessa and Beese*, 2000; *Giles et al.*, 2012). Similarly, *Wulf et al.* (2002b) assumed that a further factor for increased N_2O fluxes after slurry injection, might be the reduced air exchange and drainage conditions of the slurry from the slot into surrounding soil due to blocked soil pores through the smearing effects of injector tines.

In winter 2017 the INC and INJ treatment showed a small N_2O peak during thawing of frozen soil whereas the CON and MIN treatment did not respond. Increased N_2O fluxes from agricultural soils during freeze/thaw cycles were often reported (*Dörsch et al.*, 2004; *Singurindy et al.*, 2009; *Teepe et al.*, 2001; *Wagner-Riddle et al.*, 2007). We suggest that the increased N_2O fluxes in the INC and INJ treatment were caused by a combination of substrate release for denitrification by mechanical breakdown of aggregates by freezing (*Dörsch et al.*, 2004) and growth of microbial biomass activated through the additional C source from the slurry (*Anderson and Domsch*, 1989; *Dambreville et al.*, 2006; *Mørkved et al.*, 2006). The simultaneous increase of CO_2 fluxes from these two treatments indicated a higher O_2 consumption and a higher microbial activity than in the CON and MIN treatment. In contrast to an earlier study in vegetable production from

the same experimental site (*Pfab et al.*, 2011), winter emissions contributed only a small portion of the total annual fluxes. We mainly attributed the low contribution of the winter fluxes to the annual direct N_2O emission with the short duration of the frost period (*Wagner-Riddle et al.*, 2007; *Xu et al.*, 2016).

Generally, N losses from denitrification at high NO_3^- and easily available C concentrations in soil increase with increasing GSW, where the N_2/N_2O ratio increases above a certain threshold, indicating enhanced reduction of N_2O to N_2 (*Giles et al.*, 2012). When compared to the first year, it can be assumed that the higher cumulative direct N_2O emission from the CON, MIN and INC treatment in the second year were caused by the higher soil moisture after fertilization. In contrast, annual direct N_2O emission in the INJ treatment was higher in the dryer first year. The reason for this phenomenon remains unclear. It has been shown by *Flessa and Beese* (2000) that O_2 availability after cattle slurry application was lower in the injection slot than the availability in surrounding areas or in a broadcast slurry application treatment. Thus we speculate that N_2O reduction to N_2 in the INJ treatment was higher in the second year resulting in lower cumulative direct N_2O emission.

In both experimental years, the annual direct N_2O emission in the INC and MIN treatments did not differ, although the nearly two-fold lower amount of plant-available N applied in the INC treatment than in the MIN treatment. This observation is in agreement with results from *Giles et al.* (2017) and *Meng et al.* (2005) and indicates the importance of easily available C for promoting denitrification after application of organic fertilizers (here INC treatment). However, slurry injection increased the annual direct N_2O emission significantly compared to all the other treatments in both years, which was also reflected by exorbitantly high N_2O EFs of 8.4 % (first year) and 4.4 % (second year) of the total N applied, where the MIN and INC treatment exhibited mean EFs below 2.0 %, respectively (Figure 3.3). Approximately two-fold higher EFs from cattle slurry injection compared to surface-applied cattle slurry and CAN fertilization were reported in a field study on a sandy soil (*Velthof and Mosquera*, 2011) and in an incubation experiment with a silty loam soil where the injection treatment released 3.3 % of the added cattle slurry N within 63 days after injection (*Flessa and Beese*, 2000). The corresponding EF for the surface-applied treatment was 0.2 %. The variability of EF in dependence of application technique, N type and soil texture indicates, that a more detailed categorization of the implied *IPCC* (2006) default values of 0.01 kg N_2O-N (kg N)$^{-1}$ for mineral and organic fertilization would be desirable.

3.5.2 Impact of different application techniques and incorporation management on NH_3 losses

The calculated relative NH_3 losses were in good agreement with our measured values. Thus, calculation of NH_3 emissions according to *Horlacher and Marschner* (1990)

proved to be a reliable tool for the evaluation of NH_3 losses after organic fertilizer amendments. In both years, application dependent NH_3 volatilization showed the same trends, whereas the dimension of gaseous NH_3 losses was strongly regulated by environmental factors. The strong effect of environmental factors on NH_3 release has also been reported by *Mattila* (2006); *Sommer et al.* (2003); *Sommer and Hutchings* (2001) and *Sommer and Olesen* (2000). Thus, the comparison of NH_3 volatilization between different studies may become difficult if environmental conditions are not comparable.

Results from banded slurry application without incorporation were in accordance with the results from *Horlacher and Marschner* (1990) and *Sommer and Olesen* (2000) and showed that, given bare soil, surface application without incorporation is only an alternative for farmers, if infiltration is guaranteed through rainfalls immediately after application. This result also verifies the current German legislation which prohibits leaving slurry on the surface of bare soils (*DüV*, 2017).

Due to direct injection and therewith connected low contact time of the slurry with the atmosphere, INJ treatment showed the lowest NH_3 volatilization of all treatments. However, in the second experimental year, the reduction of NH_3 emission after slurry injection was on the same level as in the INC treatment when slurry was incorporated within the first 2 h after application. We assume that the similar NH_3 losses in these two treatments were mainly caused by a lower air temperature after application. The lower air temperature reduced the potential for NH_3 volatilization and thus the risk of NH_3 losses until the slurry incorporation. Furthermore, the dry top soil led to agglomeration and resulted in an incomplete closure of the injection slot in the INJ treatment. Through the increased pore volume, the exchange interface with the atmosphere might have additionally increased NH_3 losses from INJ treatment.

Rising NH_3 emissions were measured with increasing duration between surface application and incorporation, where NH_3 losses decreased exponentially. This was also reported by *Sommer and Hutchings* (2001) and *Wulf et al.* (2002a), and it clearly highlights the necessity of a prompt incorporation after surface application.

When compared to the INJ treatment in the first year, NH_3 emission was higher in the INC treatment. This might be attributed to the environmental conditions before incorporation and shows the uncertainties of this application technique because the risk of NH_3 volatilization can be particularly high immediately after surface application. Consequently, slurry injection provides enhanced security compared to delayed incorporation with regard to NH_3 losses. Similarly, *Mattila* (2006) concluded, that immediate incorporation or injection are suitable techniques for reducing NH_3 losses, whereas the injection reduced the losses more efficiently.

3.5.3 Influence of application technique and fertilizer form on maize yield and N removal

In accordance with the results from *Gutser et al.* (2005) the MIN treatment showed the highest N removal in both years due to a nearly two-fold higher amount of inorganic N applied when compared to the slurry fertilized treatments (refer to Table 3.1). The MFE of the INJ treatment agreed with the assumed percentage of plant-available N for cattle slurry with 50 % (*DüV*, 2017, 2006). In contrast, the mean MFE of the INC treatment over both years was only 33 % indicating a substantially lower N availability for maize plants.

Contrary to the assumption of increased N availability for plants with decreasing gaseous N losses (*Webb et al.*, 2010), the results from the present study tended to show the opposite (comparison of gaseous N emissions and N removal in the INJ and INC treatments). This might be due to further, non-detected N losses (such as *e.g.* N_2). Lower yields and N removals from broadcast slurry application with subsequent incorporation compared to slurry injection were also reported from *Federolf et al.* (2016) and from *Sutton et al.* (1982). Both studies attributed the higher N removal in the injection treatment to better nutrient availability near the maize roots in the injection slots, whereas nutrients in the INC treatment were more distant from the roots which might have led to a reduced N uptake especially under low soil moisture conditions (*Mattila*, 2006). Moreover, higher soil slurry interactions and a following higher N immobilization through broadcast slurry fertilization were reported from *Sørensen and Amato* (2002).

The clearer difference between the CON treatment and all fertilized treatments in the second experimental year was mainly caused by the lower initial N_{min} content of the soil. In contrast N removal of each fertilized treatment was quite similar within the two experimental years, which was mainly a result of the appropriate amounts of N applied in dependence of the initial N_{min} level. In the second year, the DM yields of the fertilized treatments were marginally increased compared to the first, probably because of a higher amount of precipitation during the vegetation period. Whereas a dryer period, shortly before harvest in 2016, decreased the FM yields of all treatments.

3.5.4 Effect of application technique and fertilization on CO_2 footprint

Area- and yield-related GHG emissions were influenced differently through fertilization and application technique. Direct N_2O emission contributed the major part of the total annual GHG emission (59 % - 91 % over all treatments and years). For the first year, our data from the MIN treatment were well in agreement with the results from studies on silage maize cultivation, fertilized with mineral N (*Camargo et al.*, 2013; *Jacobs et al.*, 2017). In contrast to these studies, the release of N_2O and thus GHG emission was substantially higher in the injection treatment. The main reason for this phenomenon was the discrepancy between the EFs used by *Camargo et al.* (2013) and by *Jacobs et*

al. (2017) (default value: 1 % (*IPCC* 2006)) and our measured EFs. A higher N_2O EF from injection treatment compared to slurry incorporation was also observed by *Wulf et al.* (2002b). However, total area-related GHG emission over all tested treatments from *Wulf et al.* (2002b) was on a considerably lower level, as in the present study, probably due to the shorter observation period of 42 days and consequently the highest share in total CO_2 equivalents from incorporation treatment was therefore attributed to NH_3 losses.

Based on the observations from the present study, N_2O emission and thus application technique has the highest reduction potential of total GHG emissions from maize production and should be the main target to improve the CO_2 footprint from silage maize systems.

For the organically fertilized treatments, fuel consumption had the second highest proportion of the total GHG emission. Although the absolute fuel consumption was highest in the INJ treatment, the proportion of fuel consumption to the total GHG emission in this treatment was lowest. The share of net CH_4 uptake or emission on total GHG emissions was marginal.

3.6 Conclusions

With regard to the atmospheric burden, trail hose application with immediate harrowing (INC) seemed to be the most recommendable application technique for cattle slurry on the loamy clay soil representative for our experimental region and thus, the best management practice for farmers to reduce GHG emissions during silage maize production. Moreover, the sum of gaseous N losses (N_2O, NH_3) and leaching losses (NO_3^-) was lower in the INC treatment compared to INJ treatment where the N removal from these two treatments did not differ in either year. A further NH_3 reduction could probably be achieved through direct incorporation during the application of the slurry by dragging a harrow on a trail hose applicator device.

Mineral fertilization with CAN had a higher total GHG release compared to INC treatment, whereby the area-related direct N_2O emission did not differ. Nevertheless, direct N_2O emission contributed 65 % of the total GHG emission in this treatment and therefore measures that reduce direct N_2O emission in the field would also efficiently help to reduce total GHG emission from farms without livestock, *e.g.* for the production of silage maize for biogas plants.

Slurry injection turned out to be the suboptimal application technique at our experimental site with a silty loamy texture resulting in exorbitantly high GHG release, mainly induced by high direct N_2O emission. Nitrification inhibitors (NIs) were shown to re-

duce N_2O emission efficiently (*Akiyama et al.*, 2010; *Ruser and Schulz*, 2015). Therefore, further studies should focus on the potential of NIs in reducing N_2O emissions from slurry injection to optimize this application technique.

Additionally, further studies measuring direct annual N_2O emission covering a broad range of study sites should be conducted to evaluate application technique-specific and site-specific N_2O EFs to reduce the uncertainty of the IPCC default value.

3.7 Acknowledgments

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4 Effect of nitrification inhibitors on N_2O emissions after cattle slurry application

4.1 Abstract

Cattle slurry injection has often been shown to be an efficient measure for reducing ammonia (NH_3) losses from soils, however it could also increase nitrous oxide (N_2O) emissions, which may then contribute the most to total greenhouse gas (GHG) release in silage maize production. Nitrification inhibitors (NIs) have frequently been reported to be an efficient tool for reducing the release of N_2O. Since the chemical and physical properties of commercially available NIs vary, it can be assumed that they also differ in their N_2O inhibition potential. This study therefore tested the effect of different NIs added to cattle slurry before injection on N_2O fluxes from a Haplic Luvisol under silage maize production in Southwest Germany. Dependent on the weather conditions in the two experimental years, annual N_2O emission varied between 16.2 and 11.5 kg N_2O-N ha^{-1} yr^{-1} when slurry was injected (INJ), and between 2.1 and 6.7 kg N_2O-N ha^{-1} yr^{-1} when broadcast slurry was incorporated (INC). The use of NIs resulted in a 36 % reduction in mean annual N_2O emissions from slurry injection over both years. With non-significant reductions in N_2O emissions from slurry injection through the addition of NIs in the first year and a strong and significant reduction in the second year, NIs decreased the mean fertilization-induced N_2O and total GHG release in both years by an order of magnitude comparable to slurry incorporation. Thus alongside slurry incorporation, slurry injection combined with NI application proved to be an equally appropriate fertilization strategy in terms of the atmospheric burden under the given conditions. Compared to single application of 3,4-dimethylpyrazole phosphate (DMPP) or 3,4-dimethylpyrazole succinic acid (DMPSA), the combination of these two NIs (DMPP & DMPSA) tended to have the most beneficial effect on reducing N_2O and total GHG release in both years. This effect was caused by a greater reduction in N_2O, probably due to a combination of the earlier (DMPP) and delayed (DMPSA) release behavior of the active compound dimethylpyrazole (DMP), resulting in prolonged ammonium (NH_4^+) stabilization. In an additional treatment, the effect of NIs plus slurry incorporation was investigated in the second year only. Here, there was a tendency for NIs to reduce the annual N_2O release by 20 % in comparison to slurry incorporation alone. Maize yield and N removal were not affected by any of the NIs investigated, probably due to an exceedance of the optimal N doses masking the beneficial effect of NIs.

4.2 Introduction

Techniques for applying organic fertilizers can have an immense impact on direct N_2O and NH_3 emissions from agricultural soils (Chapter 3; *Webb et al.*, 2010). These two

gaseous N losses (N_2O, NH_3) influence global warming, have harmful environmental effects and decrease the nitrogen use efficiency (NUE) of cropping systems (*Cassman et al.*, 2002; *Mosier*, 2001). Besides the objective of reducing gaseous N losses to improve the internal N cycle and avoid the extra cost of N-fertilizers (*Dawson et al.*, 2008), a reduction in N losses and fuel consumption that influence the GHG release from cropping systems has been declared a national and international political goal to protect the earth's climate (*Flessa et al.*, 2002; *UBA*, 2017). Slurry injection into the soil has been shown to be an efficient way of reducing NH_3 emissions (*Mannheim et al.*, 1995; *Sommer and Hutchings*, 2001), whereas slurry incorporation following surface application always carries the risk of increased NH_3 emission prior to incorporation of the slurry (Chapter 3; *Sommer and Hutchings*, 2001; *Wulf et al.*, 2002a), especially in conditions of high air temperature, wind velocity and solar radiation (*Horlacher and Marschner*, 1990; *Sommer et al.*, 2003).

In contrast, slurry injection can promote anaerobic conditions in the injection slot and thus stimulate N_2O release from denitrification (*Comfort et al.*, 1990; *Flessa and Beese*, 2000).

As descripted in the study of the preceding section (Chapter 3), which was conducted simultaneously and at the same study site as the present study, the injection of cattle slurry under silage maize cultivation on a Haplic Luvisol in Southwest Germany led to considerably higher direct nitrous oxide (N_2O) emissions than those from broadcast slurry incorporation. These high N_2O emissions from injection exceeded the N amount lost as NH_3 after incorporation. Thus slurry injection resulted in significantly higher total GHG releases over the two years (including N_2O, NH_3, nitrate (NO_3^-) losses and fuel consumption from the particular application technique). However, weather-dependent high NH_3 losses following slurry incorporation decreased its reductive effect on the GHG balance.

There is consequently still a requirement for a fertilization strategy that reduces both NH_3 and N_2O losses efficiently in order to decrease the atmospheric burden of silage maize production with organic fertilization. The use of nitrification inhibitors (NIs) has often been shown to be an efficient technique for decreasing N_2O emissions after application of N-fertilizers rich in NH_4^+ or fertilizers that release substantial amounts of NH_4^+ such as urea (*Akiyama et al.*, 2010; *Ruser and Schulz*, 2015).

Ammonia monooxygenase (AMO) is the first enzyme that is involved in the oxidation of NH_4^+ to NO_3^- (*Arp et al.*, 2002). The inhibition of AMO by NIs directly decreases the nitrification rate and reduces the concentration of NO_3^-, which serves as a substrate for denitrification (*Subbarao et al.*, 2006). Hence, the two main pathways of N_2O production (*Bateman and Baggs*, 2005) in soils are blocked or the strength of their source at

least decreased. The additional supply of easily available carbon (C) from organic fertilizers means that there is a great risk of promoting denitrifying conditions due to enhanced oxygen (O_2) consumption through microbial respiration (*Baggs et al.*, 2000; *Smith*, 1980) and providing electrons that are essential for C heterotrophic NO_3^- reduction (*Weier et al.*, 1993). Thus, NIs would seem to be an appropriate tool for N_2O reduction since they desynchronize C and NO_3^- availability following slurry application. The reduction of direct N_2O emission through NIs could therefore be of interest for systems with surface application and subsequent incorporation, in particular those systems with slurry injection.

Few studies up to now have confirmed a significant reduction in N_2O emission through the addition of NIs to liquid manure (*e.g. Dittert et al.*, 2001; *Vallejo et al.*, 2005), and knowledge on the effect of NIs in liquid manures is still limited with regard to N_2O release. This restricted knowledge is due in particular to the limited numbers of NIs within a study and a lack of full annual measurements. Moreover, the effectiveness of new NIs is not well known.

NIs differ in their chemical and physical characteristics, such as water solubility, volatility, sorption properties and decomposition rates in soils. Consequently, the effectiveness and persistence of their particular active compounds are influenced differently by varying environmental and soil conditions (*Subbarao et al.*, 2006). A comparison of the effectiveness of different NIs between studies remains difficult. Furthermore, the observation periods of previous investigations have varied and have mainly been less than one year. At the present study site in Southern Germany, it has been shown that annual N_2O measurements are necessary for reliable estimations of N_2O emissions (*Jungkunst et al.*, 2006; *Kaiser and Ruser*, 2000; *Pfab et al.*, 2012).

The aim of the present study was to determine the effectiveness of differing and partly new or not yet marketed NIs on N_2O reduction after cattle slurry injection and/or incorporation within a two-year study with full annual measurements. Furthermore, the impact of NIs on silage maize yield, N removal and total GHG release from silage maize production were determined in the field to enable an integrated evaluation of slurry injection and/or incorporation combined with NIs. The differing NIs investigated in the present study are briefly described below to contribute to an understanding of the differences in their effectiveness at reducing N_2O.

DMPP, also known under the trade name of ENTEC®, has been intensively used and tested in agriculture in the last decade and has proved to be an appropriate NI due to having a mobility in soils similar to NH_4^+ and strongly inhibiting NH_3 oxidation (*Azam et al.*, 2001; *Subbarao et al.*, 2006; *Zerulla et al.*, 2001). *Wissemeier* (pers. comm.) has assumed that one mode of action of the NI DMPP, which belongs to heterocyclic N

compounds with two adjacent ring N atoms, is related to Cu chelation. In the present study, a novel liquid formula of DMPP was used, bearing the trade name ENTEC® FL, which enables the use of DMPP with slurry and digestates (*EuroChem Agro*, 2014).

To the best of the authors' knowledge, the effectiveness of the new NI DMPSA (CA 2933591 A1 2015/06/18) has never previously been tested in combination with cattle slurry. This new inhibitor combines the generally known inhibitory effect of DMP (*McCarty and Bremner*, 1989) with the release behavior of the organic acid succinic acid to retard the inhibitory effect (*Guardia et al.*, 2018; *Pacholski et al.*, 2016).

A combination of DMPP and DMPSA may result in a more efficient reduction of N_2O release than the single NI alone due to an immediate DMP release after slurry application from DMPP and a prolonged NH_4^+ stabilization subsequently through a preliminary persistence of DMPSA.

The reactive compound 2-Chloro-6-(trichloromethyl)-pyridine, better known as Nitrapyrin, is a substituted heterocyclic N compound that is highly soluble in organic solvents (*McCarty and Bremner*, 1989). It is generally assumed that the main mode of AMO inhibition is a Cu-chelating effect of the active compound and is caused by the Cl and CCl_3 group substituted on the C atom adjacent to the ring N atom (*McCarty*, 1999). Furthermore *Vannelli and Hooper* (1992) have observed a non-competitive as well as an apparently weak mechanism-based inhibition of AMO by Nitrapyrin.

Since Nitrapyrin bears a risk of volatilization caused by a relatively high vapor pressure (*Subbarao et al.*, 2006), there has been a critical appraisal of the release of Nitrapyrin as an organic chlorine compound into the environment (*Trenkel*, 1997; *Zerulla et al.*, 2001). The new formulation of Nitrapyrin as N-LOCK™ Nitrogen Stabilizer, listed in Germany since 2015 (*Deutscher Bundestag*, 2017), should prevent the volatilization of Nitrapyrin because it comes as a capsule suspension (*The Dow Chemical Company*, 2012). This capsule suspension means that a prolonged release of the active compound is conceivable and there is increased interest in investigating this newly traded inhibitor recommended as an additive for slurry and digestates.

The solution of the two pyrazole derivatives 1,2,4 Triazole (TZ) and 3-Methylpyrazole (MP), marketed under the trade name PIADIN®, is also suitable as an additive for slurry and digestates (*SKW Stickstoffwerke Pisteritz GmbH*, 2007). This NI contains two active substances (TZ and MP) that inhibit nitrification separately (*McCarty and Bremner*, 1989). Both active compounds belong to the heterocyclic N compounds and have two adjacent N atoms, which have been reported to have an especially strong inhibition effect (*McCarty*, 1999). However, the results reported in previous studies regarding their effectiveness at reducing N_2O release are controversial (*Barneze et al.*, 2015; *Hu et al.*,

2013; *Spott et al.*, 2015; *Wolf et al.*, 2014), probably due to the varying experimental conditions and differing observation periods.

The active substance DCD (dicyandiamide) was detected as an NI in the early 1920s (*Prasad et al.*, 1971), carries the brand name Alzon® and is suitable for use with animal slurries (*SKW Stickstoffwerke Pisteritz GmbH*, 2012). This well-known NI, whose effectiveness has repeatedly been confirmed (*Amberger*, 1989; *Di and Cameron*, 2004, 2002; *Merino et al.*, 2002; *Vallejo et al.*, 2005), has functioned primarily as a benchmark for appropriately evaluating the effectiveness of the remaining NIs. The main inhibition mechanism of DCD is again its Cu-chelating effect (*Amberger*, 1989, 1986). DCD is a water-soluble, non-volatile N stabilizer (*Amberger*, 1989), which carries the risk of being easily leached out of the root zone due to its several-fold greater mobility than NH_4^+ (*McCarty and Bremner*, 1989; *Pasda et al.*, 2001), especially in saturated light-textured soils with a strong downwards-directed water flow (*Ruser and Schulz*, 2015; *Zerulla et al.*, 2001).

The present study was performed to verify the following hypotheses: (i) NIs delay the availability of NO_3^- after slurry application and therefore reduce N_2O emissions, (ii) different NIs have different N_2O reduction potentials in the short- and long-term due to their varying chemical and physical characteristics together with slurry application, and (iii) slurry application with NIs strongly reduces field-related GHG release compared to slurry application alone.

4.3 Material and methods

4.3.1 Study site

The experiment was conducted at the University of Hohenheim's Heidfeldhof research station (48°43'0.30'' N; 9°11'30.64'' E; 404 m a.s.l.). The soil has a silty loamy texture (2 % sand, 74 % silt, and 24 % clay) and is classified as Haplic Luvisol. The study site is characterized by long-term mean precipitation of 691 mm yr^{-1} and an annual mean air temperature of 10.1 °C (*Agrarmeteorologie Baden-Württemberg*, 2017). The initial pH in the top soil layer (0-30 cm depth) is 7.0 (10^{-2} *M* $CaCl_2$). The C_{org} and N_t content of the A_p horizon are 0.72 % and 0.10 % respectively. At the start of the experimental period, the initial mineral N (N_{min}) content (0-60 cm soil depth) was 44 and 21 kg N ha^{-1} in the first and second year respectively.

4.3.2 Experimental design and crop management

In the first year, data were collected between 28 April 2015 and 19 April 2016. In the second experimental year, measurements were carried out on an adjacent field between 28 April 2016 and 3 May 2017. A fully randomized block design was established in

both years with four replicates and a plot size of 10 m x 3 m. The treatments investigated are listed in Table 4.1.

The results of unfertilized control (CON), mineral fertilization with calcium ammonium nitrate (MIN), cattle slurry incorporation (INC), and slurry injection (INJ) (treatments i-iii and v, Table 4.1) were taken from Chapter 3. In the present study, additional results from slurry incorporation with NI and slurry injection with different NIs, as listed in Table 4.1 (treatments iv; vi-xi) are shown. To ensure a comparison of the application techniques with and without NI, as well as enabling background emissions to be estimated, reference will also be made to the results of the INC, INJ and CON treatments described in Chapter 3. Except for the treatment INJ + TZ & MP (tested only in the first experimental year) and the treatment INC + DMPP & DMPSA (tested only in the second experimental year), all remaining treatments were tested in both experimental years.

Depending on the treatment, the slurry was surface-applied with a trail hose applicator and incorporated using a circular harrow after 2 h (working depth 15 cm) (INC) or injected with an injecting cultivator, placing the slurry in an injection slot at 15 cm soil depth (share width 5 cm) (INJ). The space between trail hoses or injector tines was 75 cm. Before fertilization, a homogenous mixture of the particular NI and 0.8 m^3 slurry was ensured by mixing both components for 15 min with a rotary piston pump with a cutter (Vogelsang, R116-60S, pump capacity 0.63 m^3 min^{-1}).

Based on total N, 170 kg N ha^{-1} was applied in all fertilized treatments in the first year. This corresponded to the maximum amount of organic N-fertilizer permitted under German legislation about N-fertilization (*DüV,* 2006). To ensure greater compatibility between the two experimental years, 190 kg total N ha^{-1} was applied due to the lower initial N_{min} content in soil in the second year. In the first experimental year, cattle slurry was applied in two doses. At the first fertilization (10 May) approximately 80 % of the total N was applied. The second cattle slurry dose (18 June) was surface-applied by trail hose instead of injection or incorporation in order to avoid damage to the growing maize plants in 2015. NIs were only applied with the first N dose in the cattle slurry treatments. In the second experimental year, N-fertilizer was applied in full in one dose (3 May). Slurry was characterized at the first and second fertilizations in 2015 as having a total N content of 3.1 and 2.8 kg m^{-3}, a total NH_4^+-N content of 1.3 and 1.4 kg m^{-3}, a dry matter (DM) content of 8.5 and 7.2 % and a pH of 6.6 and 6.6 respectively. In 2016, the total N content of cattle slurry was 3.7 kg m^{-3}, the total NH_4^+-N content was 2.2 and the pH was 7.3. Maize (*Zea mays* L., variety "Amadeo") was sown in both years (12,700 plants ha^{-1}) with a sowing depth of 5 cm and a row distance of 75 cm. In 2015, maize was sown on 13 May and whole shoots harvested on 26 August as silage maize. In 2016, maize was cultivated on 4 May and harvested on 13 September.

Table 4.1: Abbreviation and description of the different treatments with N-fertilizer form, application technique, nitrification inhibitor added (active substance(s)) and trade name) and sampling period.

	Treatment		N-fertilizer	Application technique	Nitrification inhibitor	Trade name	Sampling period		Comment
							1st yr	2nd yr	
i	**CON**		unfertilized	-	-	-	X	X	see Chapter 3
ii	**MIN**		CAN[1]	broadcast	-	-	X	X	see Chapter 3
iii	**INC**		cattle slurry	trail hose + incorporation	-	-	X	X	see Chapter 3
iv	**INC**	**+ DMPP &**	cattle slurry	trail hose + incorporation	3,4-dimethylpyrazole phosphate	ENTEC® FL		X	
		DMPSA			3,4-dimethylpyrazole succinic acid	currently not traded			
v	**INJ**		cattle slurry	injection	-	-	X	X	see Chapter 3
vi	**INJ**	**+ DMPP**	cattle slurry	injection	3,4-dimethylpyrazole phosphate	ENTEC® FL	X	X	
vii	**INJ**	**+ DMPSA**	cattle slurry	injection	3,4-dimethylpyrazole succinic acid	currently not traded	X	X	
viii	**INJ**		cattle slurry	injection	3,4-dimethylpyrazole phosphate	ENTEC® FL	X	X	
		+ DMPP & DMPSA			3,4-dimethylpyrazole succinic acid	currently not traded			
ix	**INJ**	**+ Nitrapyrin**	cattle slurry	injection	nitrapyrin	N-LOCK™ Nitrogen Stabilizer	X	X	
x	**INJ**	**+ DCD**	cattle slurry	injection	dicyandiamide	Alzon®	X	X	
xi	**INJ**	**+ TZ & MP**	cattle slurry	injection	1,2,4 Triazole & 3-Methylpyrazole	PIADIN®	X		

[1]calcium ammonium nitrate

In both years, the seedbed was prepared with a cultivator one week before sowing. After harvesting, the maize stubbles were mulched and subsequently incorporated with a cultivator. During the remaining observation period, the field lay fallow without any further soil cultivation activity or crop management practice.

4.3.3 Nitrification inhibitors and application rates

The applied amount of NIs tested in the present study was at least in the amounts recommended by the producers.

For treatments with DMPP, a quantity of 6 L ha^{-1} was used of the liquid formula ENTEC® FL (containing 25-50 % ammonium nitrate and 1-5 % of the active compound DMPP) (*EuroChem Agro*, 2014). Despite the producer recommending a lower amount (4 L ha^{-1}) for slurry injection compared to broadcast incorporation (6 L ha^{-1}), the same amount was used for both application techniques to guarantee comparability.

For DMPSA, the same amount of DMP as in the DMPP treatment was used.

For the treatments with the combination of the two active compounds DMPP and DMPSA, half of the above-mentioned amounts of each NI were added to the slurry.

Nitrapyrin as N-LOCK™ Nitrogen Stabilizer was tested in the present study using the amount of 2.5 L ha^{-1} (*The Dow Chemical Company*, 2012).

For the treatment with the solution of the two pyrazole derivates (containing 3.00-3.25 % TZ; 1.50-1.65 % MP), marketed under the trade name PIADIN®, a quantity of 6 L ha^{-1} was applied (*SKW Stickstoffwerke Pisteritz GmbH*, 2007).

DCD was added to the slurry in pure form using 102.3 g DCD m^{-3} $slurry^{-1}$, equivalent to the 12.5 times amount of DMPP.

4.3.4 Trace gas sampling and flux calculation

During the two experimental years, N_2O and CO_2 fluxes were measured at least weekly in the morning using the closed chamber method (*Hutchinson and Mosier*, 1981) with a circular chamber design (inner diameter of 30 cm). For the particular treatment, the base frames were positioned in a way that was representative for the area, for example 7 % of it covering the slot zone and 93 % covering the area next to the slot for INJ treatments (Chapter 10.2; *Gassner*, 2017). At each trace gas sampling, four samples were taken from each chamber at 15-20 min intervals, depending on the expected heights of the trace gas fluxes. The first sample was taken immediately after the chamber was closed. The chamber design and sampling procedure are described in detail by *Pfab et al.* (2011). The samples of trace gas concentrations were measured gas chromatographically (GC 450 Greenhouse Gas Analyzer, Bruker Daltonic, Bremen, Germany). N_2O and CO_2 flux rates were calculated using the package "gasfluxes" (*Fuss and Asger*,

2014) for R software (*R Core Team*, 2016), which selects the most suitable model for calculating trace gas fluxes, including numerous factors, which are described in detail by *Ruser et al.* (2017).

The N_2O emission factor (EF) related to total N applied was calculated as described by *Velthof and Mosquera* (2011) (Equation 4.1):

Equation 4.1

$$EF = \frac{(N_2O - N\ emission_{fertilized\ treatment} - N_2O - N\ emission_{CON})}{total\ N_{fert}}\ x\ 100$$

where EF is the emission factor of total N applied [%], N_2O-N emission is the mean cumulative direct N_2O-N emission of the particular experimental year either from the treatments fertilized ($emission_{fertilized\ treatment}$) or from the unfertilized control ($emission_{CON}$) [kg N_2O-N ha^{-1} yr^{-1}], and total N_{fert} is the amount of total N-fertilizer applied as cattle slurry [kg N ha^{-1} yr^{-1}].

Fertilization-induced N_2O-N emissions from fertilized treatments were corrected for "unavoidable" field-related N_2O-N release attributed to the background emission (CON) of the respective year from silage maize production.

4.3.5 Weather data, soil & plant sampling and laboratory analyses

Weather data were provided by the university's meteorological station, which is located 500 m from the study site. Soil temperature at a depth of 5, 10 and 15 cm was detected throughout the observation period separately in each block (n=4) using data loggers (Logtag, TRIX-8, CIK solutions, Karlsruhe, Germany).

Simultaneously to gas sampling, soil sampling (0-30 cm) was conducted to determine the N_{min} content (0.5 *M* K_2SO_4) and soil moisture status. As described in Chapter 3, except for the INJ treatments, three samples were taken randomly with an auger from each plot. These soil samples from the four replicated plots of each treatment were then pooled and stored frozen before analyses. For the INJ treatments, soil samples were taken directly above the injection slot and separately in the area between the slots. To identify the initial N_{min} contents, additional soil samples were taken from each plot separately before sowing (0-30 cm, 30-60 cm, and 60-90 cm).

To determine the N_{min} content of the soil samples, the extraction procedure and laboratory analyses were performed in the same way as described in Chapter 3.3.5, using 20 g of fresh soil for extraction with 80 mL of a 0.5 *M* K_2SO_4 solution. The NO_3^--N and NH_4^+-N concentrations in the samples were measured using flow injection analyses (3 QUAAtro.AQ2.AACE, SEAL Analytical, UK). A further aliquot of each soil sample was dried at 105 °C for 24 h to determine soil moisture gravimetrically.

In both years, fresh matter (FM) yield was detected with a maize chopper during harvest. For this, the two central maize rows were chopped. An aliquot of each plot was taken, dried and used for DM yield calculation. Additionally, the C and N contents of plant samples were measured with an elemental analyzer (vario MAX CN, Elementar Analysensysteme, Hanau, Germany) to calculate N removal.

4.3.6 Calculation of CO_2 equivalents

To enable a comparable evaluation between different cattle slurry application techniques (incorporation, injection) with and without NI, as well as the effectiveness of the particular NI at reducing the atmospheric burden, the gaseous N losses (N_2O, NH_3), leaching losses (NO_3^-) and fuel consumption were summed after conversion into CO_2 equivalents. The calculation of CO_2 equivalents was performed as described in Chapter 3.3.4 using the default GWP of 298 g g^{-1} CO_2 for N_2O (*IPCC*, 2007) as conversion factors. Fertilization-induced GHG balances from fertilized treatments were corrected for "unavoidable" field-related CO_2 equivalents attributed to basic emission (CON) for the respective year from silage maize production.

To estimate the indirect contribution of NH_3 emission to total GHG release, the same NH_3 emission as for the INC and INJ treatment in Chapter 3.4.4 were assumed for the respective INC or INJ treatment with NI.

To quantify the amount of NH_3 re-emitted as N_2O-N, an emission factor (EF) based on N_2O-N emission from a mineral-fertilized treatment was used, which was conducted at the same site and at the same time as the treatments in the present study (Chapter 3.4.3).

Equation 11.10 from the *IPCC* (2006) guidelines was used to calculate indirect N_2O emissions *via* NO_3^- leaching in the same way as described in Chapter 3.3.4. Data from the field experiment were used to calculate the amount and N content of aboveground harvest residues (stubble, leaves), assuming that 10 % of silage maize harvested remained in the field (*Jacobs et al.*, 2017). For belowground harvest residues, the *IPCC* (2006) default values for maize were used. Indirect emission as a result of soil N mineralization and C-stock changes were not considered.

Diesel consumption for tillage, sowing, harvesting and the different fertilizer application techniques was also calculated identically to the description given in Chapter 3.3.4, using the online-based calculator of the German Association for Technology and Structures in Agriculture (*KTBL*, 2009). For the calculation of site-specific fuel consumption, a distance of 1000 m was assumed between the farm and the field, the field size was defined as 1 ha and medium soil resistance was assumed. The default value of 74.1 Mg CO_2 Mg^{-1} was used to calculate CO_2 equivalents from diesel fuel, including emissions during production, transport and burning (*Juhrich*, 2016).

Emissions from the production and distribution of the NIs were excluded from the calculation of GHG release.

4.3.7 Statistical analyses

Statistical analyses were performed using the Statistical Software package SigmaStat 3.5. Data were tested for variance homogeneity. Normal distribution of residuals was tested using the Shapiro Wilk Test. N_2O fluxes from the first experimental year were log_{10}-transformed to realize variance homogeneity. A two factorial analysis of variance (ANOVA) was applied with the factors block and treatment to test the block effect in each experimental year. Owing to differences between the two experimental years (*e.g.* climatic conditions in the period after fertilization, change of experimental site) in terms of yield, N removal and cumulative N_2O emissions, a one factorial ANOVA was performed separately for each experimental year to detect differences between the treatments within the particular observation periods. Significant differences were determined using a pairwise multiple comparison procedure (*Student-Newman-Keuls, $p<0.05$, $n=4$*). The data are presented as arithmetic means with standard errors.

For datasets that did not follow a normal distribution, a Kruskal-Wallis One Way ANOVA on Ranks was chosen for statistical analysis. These were mean fertilization-induced N_2O emissions over both years (*Tukey Test, $p=0.012$, $n=8$*), yield-related CO_2 equivalents from the first year (*Student Newman Keuls Method, $p=0.007$, $n=4$*), and mean fertilization-induced CO_2 equivalents over both years (*Tukey Test, $p=0.012$, $n=8$*).

4.4 Results

4.4.1 Environmental conditions, mineral N and N_2O flux rates

The N_2O flux rates showed a high spatial and temporal variability over the two experimental years (Figure 4.1a and Figure 4.2 a). The highest flux rates of up to 6720 µg N_2O-N m^{-2} h^{-1} (INJ, first year) were observed under conditions of high NO_3^- availability and soil moisture content in the periods after fertilization.

Immediately after slurry application, the NH_4^+ content, especially in the slot areas after injection, greatly increased (Figure 4.1c and Figure 4.2c). NH_4^+ oxidation increased noticeably around four days after application and resulted in a higher NO_3^- availability for a period between 45 days (2015) and 61 days (2016) (Figure 4.1b and Figure 4.2b).

The addition of NIs resulted in a delayed and lower NO_3^- availability, as well as in lower N_2O fluxes compared to the fertilized treatments without NI (INJ or INC). Over all NI treatments, this delay lasted for around 33 days after N-fertilization in the warmer first year and for 40 days after N-fertilization in the second year, in which the mean soil

temperature (at a depth of 10 cm) was 3 °C lower in the period within 33 days of fertilization compared to the first year (Figure 4.1f).

In both years, the addition of DCD and DMPP tended to inhibit nitrification, mainly immediate after slurry injection (Figure 4.1a, b). In contrast, the addition of DMPSA and Nitrapyrin tended to have a lower inhibitory effect immediately after application, but a prolonged and greater effectiveness subsequently. The combination of the two active compounds DMPP & DMPSA inhibited nitrification in the earlier and later periods after fertilization, resulting in lower N_2O flux rates for approximately 55 days after injection when compared to the single addition of DMPP or DMPSA.

Similarly, the addition of these two active substances to the incorporation treatment (INC + DMPP & DMPSA) in 2016 showed lower N_2O flux rates for 61 days (Figure 4.2a) compared to incorporation of slurry alone (data shown in Figure 3.2).

In the unusually dry period after the first fertilization in 2015 (May to July), precipitation was 33 % lower than expected, with only one heavy rainfall event occurring approximately one month after the first fertilization (Figure 4.1f). All injection treatments showed a clear N_2O rewetting response, whereby the injection of slurry alone resulted in between 1.3 and 2.7 times higher fluxes than the injection treatments with NIs. At this N_2O rewetting peak four weeks after fertilization, injection treatments in which the NIs' main inhibitory effect was delayed (DMPSA, DMPP & DMPSA, Nitrapyrin, TZ & MP) tended to show an approximately 60 % greater reduction in N_2O emissions while NIs with a higher inhibitory efficiency in the early period (DMPP, DCD) tended to reduce N_2O fluxes less efficiently, with a reduction of around 28 % compared to injection of slurry alone.

Similarly, a heavy rainfall event approximately four weeks after the fertilization in 2016 induced high N_2O flux rates in all treatments. Again, the addition of NIs reduced the magnitude of the corresponding flux rates. However, this time the injection treatments containing NIs with an immediate inhibitory effect (DMPP, DMPP & DMPSA, DCD) tended to lead to a greater reduction of rewetting-induced N_2O fluxes (by 75 %) compared to injection of slurry alone. Inhibitors with delayed inhibition (DMPSA, Nitrapyrin) tended to show a smaller reduction in N_2O release (by 14 %).

Compared to 2015, the sum of precipitation after fertilization in 2016 was more or less in line with the long-term annual mean for precipitation and rainfall events occurred more frequently (Figure 4.1f). Consequently, more N_2O pulses were detected in the second year, during which the maximum flux rate was lower.

In the later vegetation periods, the N_{min} contents and soil moisture decreased with the development of maize plants. In both years, for the first 50 days after injection, soil moisture in the slot zones from injection treatments was higher than the soil moisture in

the INC treatments. Rainfall events resulted in higher soil moisture levels in the slot zones, even much later on (Figure 4.1e, f and Figure 4.2e).

At approximately 10 µg N_2O-N m^{-2} h^{-1}, N_2O fluxes after harvest and tillage mainly remained low. Only one freeze/thaw event in January 2017 resulted in slightly increased flux rates of up to 155 µg N_2O-N m^{-2} h^{-1} (INJ treatment), with the treatments with NI tending to show a smaller response.

CO_2 fluxes increased with rising soil temperature at 10 cm depth (r^2=0.46 in the first year and r^2=0.59 in the second year, Pearson Product Moment Correlation, $p<0.001$). They were stimulated through substrate availability after slurry application or incorporation of harvest residues (Figure 4.1d and Figure 4.2d). Additionally, the microbial respiration was positively correlated with N_2O fluxes (r^2=0.27 for the first year; r^2=0.13 for the second year, Pearson Product Moment Correlation, $p<0.001$). Moreover, soil respiration was the best predictor for the temporal variability of N_2O fluxes in the first and second experimental years across all treatments (55 % and 33 %) followed by the NO_3^- concentration in the topsoil, which accounted for another 21 % and 8 % (Stepwise Forward Regression).

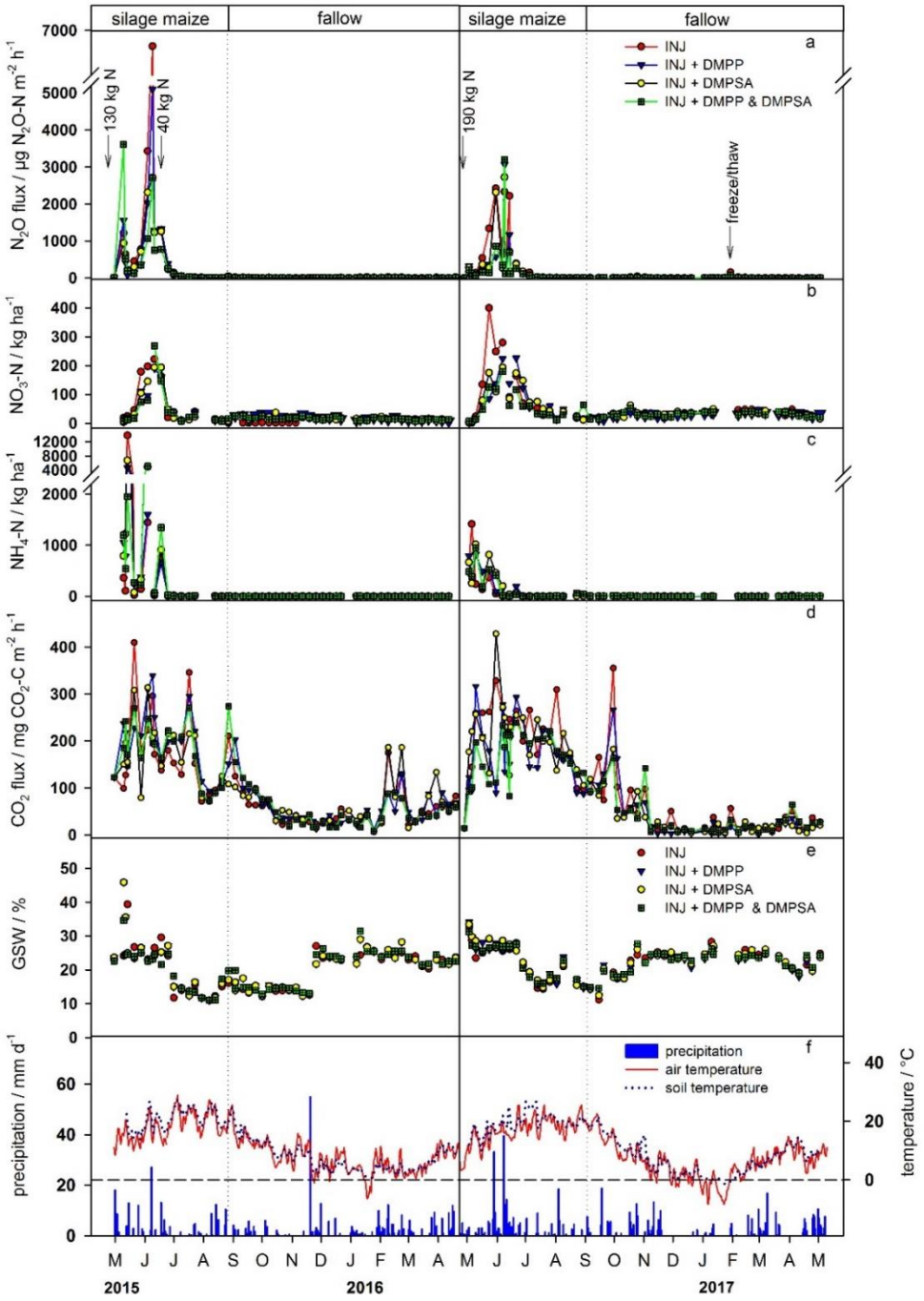

Figure 4.1: Mean N_2O flux rates (n=4) (a), NO_3^--N contents (0-30 cm) (b), NH_4^+-N contents (0-30 cm) (c), mean CO_2 flux rates (n=4) (d) and gravimetric soil water content (GSW, 0-30 cm) (e) in the slurry injection treatment without NI (INJ) and with the NIs DMPP (INJ + DMPP), DMPSA (INJ + DMPSA) and the combination of DMPP and DMPSA (INJ + DMPP & DMPSA) over the two experimental years. During cropping seasons, soil samples for N_{min} (NO_3^-, NH_4^+) and GSW determination were taken from the slot area. During fallows, soil sampling was conducted randomly from the particular plots. Arrows indicate fertilization measures. Mean air temperature (solid line), soil temperature at 10 cm depth (dotted line), and daily precipitation (black bars) (f). Error bars are omitted for the purposes of clarity. Data from the INJ treatment are taken from Chapter 3.

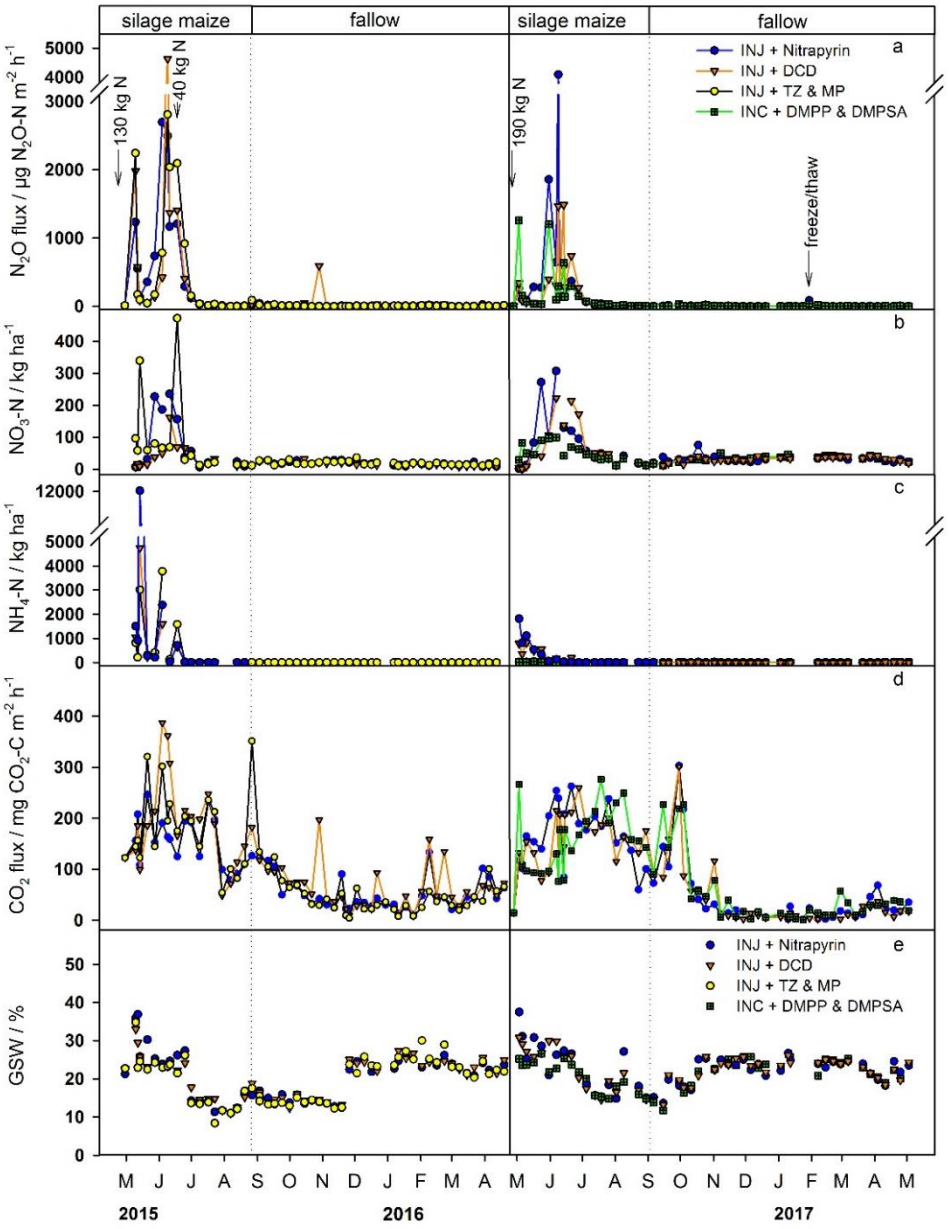

Figure 4.2: Mean N_2O flux rates (*n=4*) (a), NO_3^--N contents (0-30 cm) (b), NH_4^+-N contents (0-30 cm) (c), mean CO_2 flux rates (*n=4*) (d) and gravimetric soil water content (GSW, 0-30 cm) (e) in the slurry injection treatments with Nitrapyrin (INJ + Nitrapyrin), with DCD (INJ + DCD), with TZ and MP (INJ + TZ & MP) and the slurry incorporation treatment with the combination of DMPP and DMPSA (INC + DMPP & DMPSA) over the two experimental years. During cropping seasons, soil samples from INJ treatments for N_{min} (NO_3^-, NH_4^+) and GSW determination were taken from the slot area. During fallows, soil sampling was conducted randomly from the particular plots. Arrows indicate fertilization measures. Error bars are omitted for the purposes of clarity.

4.4.2 Annual cumulative N_2O emissions

As shown in Table 4.2 the mean annual N_2O emissions from treatments with slurry injection ranged from 4.4 kg N_2O-N ha^{-1} yr^{-1} (INJ + DCD, second experimental year) to 16.2 kg N_2O-N ha^{-1} yr^{-1} (INJ, first experimental year).

In the first year, the addition of NIs tended to reduce annual N_2O emissions from slurry injection by between 17 % and 41 %.

Table 4.2: Mean annual direct N_2O-N emissions, mean background corrected annual emission factor (EF) and background corrected fertilization-induced N_2O-N emissions averaged over both years as affected by NI addition and application technique in the first and second experimental years. Different letters indicate statistically significant differences between groups within the same column (*Student-Newman-Keuls test* for cumulative annual N_2O emission ($p<0.05$, $n=4$) and *Tukey Test* for fertilization-induced N_2O emission over both years ($p=0.012$, $n=8$)). The data from the CON, INJ and INC treatment were taken from Chapter 3.

Treatment	Cumulative N_2O emissions [kg N_2O-N ha^{-1} yr^{-1}]		EF N_2O-N in % of total N applied		Fertilization-induced N_2O emissions [kg N_2O-N ha^{-1} yr^{-1}]
	1st year	2nd year	1st year	2nd year	mean both years
CON	2.3[b]	3.3[c]			
INC	2.1[b]	6.7[bc]	0.0	1.8	1.6[b]
INC + DMPP & DMPSA	n.d.	5.4[bc]		1.1	
INJ	16.2[a]	11.5[a]	8.4	4.4	11.1[a]
INJ + DMPP	12.8[a]	5.5[bc]	6.3	1.2	6.4[ab]
INJ + DMPSA	12.4[a]	8.4[b]	6.1	2.7	7.6 [ab]
INJ + DMPP & DMPSA	9.6[a]	4.9[bc]	4.4	0.9	4.5 [ab]
INJ + Nitrapyrin	12.8[a]	7.9[b]	6.3	2.4	7.6 [ab]
INJ + DCD	11.0[a]	4.4[bc]	5.2	0.6	5.2 [ab]
INJ + TZ & MP	13.4[a]	n.d.	6.7		

CON= unfertilized control; INC= slurry incorporation; INJ= slurry injection; for NI abbreviations see Table 4.1; n.d.= not determined

In the second experimental year, the addition of all NIs resulted in a significant reduction in N_2O emissions compared to the injection of slurry alone. Here the injection treatments with NIs did not differ from broadcast slurry incorporation (INC). The addition of DMPP, DMPP & DMPSA and DCD proved to be the most efficient additives, with N_2O reduction potentials of between 52 % and 61 %. The addition of these three NIs resulted in there being no difference between N_2O emissions from slurry injection and background emissions (CON) (Table 4.2).

An additional application of DMPP & DMPSA together with broadcast incorporation tended to further decrease cumulative N_2O emissions, but not statistically significantly so.

Emission factors (EFs) varied between 1.1 and 8.4 %, with the efficient reduction in N_2O emissions through the application of NIs also reflected in the lower EFs for the NI treatments (Table 4.2). Overall, the INJ + DCD treatment exhibited the lowest EF with 0.6 % of total N applied.

Comparing the mean fertilization-induced N_2O emissions over both years, injection treatments with NIs did not significantly differ from broadcast slurry incorporation. Applying a *t-test* to separately compare the effectiveness of the particular NI when using slurry injection with injection of slurry alone, the addition of DMPP & DMPSA significantly reduced fertilization-induced N_2O emissions over both years compared to the injection of slurry alone (INJ *vs*. INJ + DMPP & DMPSA, $p=0.025$) and the use of DCD (INJ *vs*. INJ + DCD, $p=0.038$).

4.4.3 Maize yield and N removal

In both years, fresh matter (FM) yield, dry matter (DM) yield and N removal were not influenced by the addition of NIs during slurry injection (Table 4.3). The use of NIs in combination with broadcast incorporation (INC + DMPP & DMPSA) tended to decrease the yield and N removal compared to incorporation of slurry alone (INC). A correlation between gaseous N losses (NH_3, N_2O) and N removal could not be confirmed.

Table 4.3: Mean fresh matter (FM) and dry matter (DM) yields and N removal of silage maize with standard errors (SE) as affected by application technique, NI addition and experimental year. No statistically significant differences in FM yield, DM yield or N removal between treatments within the same year were detected (*Student-Newman-Keuls test, $p<0.05$, $n=4$*). Data from INJ and INC treatment were taken from Chapter 3.

Treatment	FM yield [Mg ha^{-1}]	SE	DM yield [Mg ha^{-1}]	SE	N removal [kg N ha^{-1}]	SE
2015						
INC	42.3	0.9	12.4	0.3	132	10
INJ	45.7	2.1	14.1	0.6	147	8
INJ + DMPP	49.8	1.2	12.1	1.3	143	13
INJ + DMPSA	47.6	0.7	14.2	0.3	158	2
INJ + DMPP & DMPSA	49.4	2.7	14.7	0.9	150	11
INJ + Nitrapyrin	49.7	2.1	15.1	0.9	184	19
INJ + DCD	48.7	0.9	14.7	0.5	157	7
INJ + TZ & MP	45.9	1.7	14.2	0.7	168	21
2016						
INC	33.9	0.7	14.6	0.3	120	2
INC + DMPP & DMPSA	33.0	1.0	12.9	0.6	107	7
INJ	36.4	2.6	15.4	1.4	140	11
INJ + DMPP	36.5	0.3	15.4	0.5	127	9
INJ + DMPSA	36.4	1.0	16.0	0.5	147	11
INJ + DMPP & DMPSA	37.3	1.1	15.4	0.7	124	6
INJ + Nitrapyrin	35.5	1.3	15.2	0.7	132	6
INJ + DCD	36.6	1.3	15.2	0.8	127	9

INC= slurry incorporation; INJ= slurry injection; for NI abbreviations see Table 4.1

4.4.4 CO_2 footprint

The injection treatment without NI produced the highest GHG release for silage maize production in both years (Table 4.4). Cumulative annual N_2O emissions accounted for the highest share of total GHG release in both experimental years (between 64 % and 93 %). Therefore, field-related GHG emissions followed the same order as direct N_2O emissions (see Table 4.2). Fuel consumption, NO_3^- leaching and NH_3 losses contributed a much smaller proportion to the atmospheric burden at 8 %, 5 % and 1 % respectively (means across both years).

In the first year, the use of NIs tended to reduce the CO_2 footprint for silage maize production compared to the injection of slurry alone, but not significantly so, which might be comparable to broadcast incorporation of slurry (Table 4.4).

Conversely, in the second year, all NIs investigated together with slurry injection reduced the area- and yield-related GHG release significantly compared to injection of slurry alone. This reduction resulted in a decrease in total GHG release, which was comparable to slurry incorporation. The addition of the NIs DMPP, DMPP & DMPSA and DCD did not result in a difference between slurry injection and the unfertilized control. Using NI together with slurry incorporation (INC + DMPP & DMPSA), the GHG balance also tended to be improved.

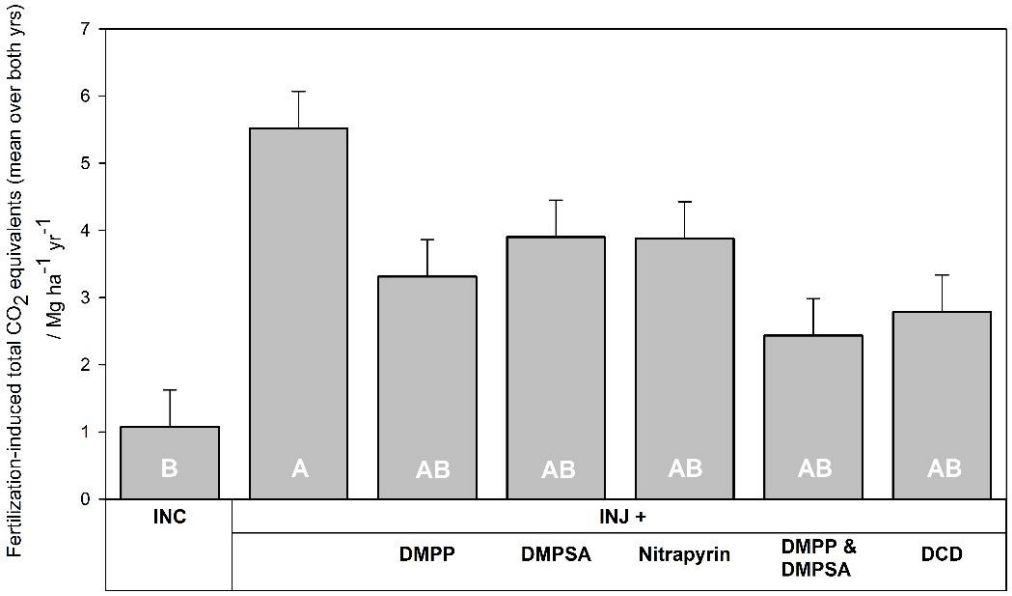

Figure 4.3: Field-related CO_2 equivalents attributed to fertilization strategy of slurry fertilized treatments (cattle slurry incorporation (INC) or injection (INJ) without/with the addition of nitrification inhibitors) in mean over both years with pooled Standard error of the mean. Fertilization-induced CO_2 equivalents were corrected for total CO_2 equivalents from CON treatment. Different letters indicate statistically significant differences between groups (*Tukey Test, p=0.012, n=8*). The data from CON, INC and INJ treatment were taken from Chapter 3.

Table 4.4: Mean area- and yield-related greenhouse gas (GHG) emissions and GHG sources as affected by application technique, NI addition and experimental year. Values in brackets indicate the percentage of the single sources to total GHG emission. Different superscript letters indicate statistically significant differences between treatments within the same year and column (*Student Newman Keuls* for dataset from first (*ANOVA on Ranks*; *p=0.007*, *n=4*) and second year (*ANOVA*; *p<0.05*, *n=4*)). Data from CON, INJ and INC treatment were taken from Chapter 3.

Year	Treatment	GHG sources								Area-related GHG emissions	Yield-related GHG emissions
		N_2O emissions		NH_3 emissions		NO_3^- leaching		Fuel consumption			
		[kg CO_2 eq. ha^{-1} yr^{-1}] (percentage of single source to total GHG emission)								[kg CO_2 eq. ha^{-1} yr^{-1}]	[kg CO_2 eq. Mg^{-1} DM^{-1}]
1st	CON	1067.1	(82.2)	0.0	(0.0)	32.2	(2.5)	198.9	(15.3)	1298.2^{b}	106.9^{b}
	INC	982.1	(64.1)	65.1	(4.3)	213.3	(13.9)	272.0	(17.7)	1532.4^{b}	123.8^{b}
	INJ	7598.8	(93.2)	45.5	(0.6)	217.7	(2.7)	288.9	(3.5)	8150.9^{a}	570.1^{a}
	INJ + DMPP	5988.3	(91.6)	45.5	(0.7)	214.0	(3.3)	288.9	(4.4)	6536.7^{a}	563.1^{a}
	INJ + DMPSA	5805.5	(91.3)	45.5	(0.7)	219.0	(3.4)	288.9	(4.5)	6358.9^{a}	450.2^{a}
	INJ + DMPP & DMPSA	4508.4	(89.1)	45.5	(0.9)	219.0	(4.3)	288.9	(5.7)	5061.8^{a}	338.8^{a}
	INJ + Nitrapyrin	6002.2	(91.5)	45.5	(0.7)	223.3	(3.4)	288.9	(4.4)	6559.8^{a}	427.8^{a}
	INJ + DCD	5146.0	(90.3)	45.5	(0.8)	219.7	(3.9)	288.9	(5.1)	5700.1^{a}	386.4^{a}
	INJ + TZ & MP	6277.8	(91.9)	45.5	(0.7)	220.1	(3.2)	288.9	(4.2)	6832.3^{a}	505.6^{a}
2nd	CON	1543.4	(87.7)	0.0	(0.0)	25.85	(1.5)	191.2	(10.9)	1760.5^{C}	164.3^{B}
	INC	3128.5	(85.0)	58.6	(1.6)	236.72	(6.4)	254.9	(6.9)	3678.7^{BC}	251.5^{B}
	INC + DMPP & DMPSA	2536.0	(82.3)	58.6	(1.9)	232.5	(7.5)	254.9	(8.3)	3082.0^{BC}	243.0^{B}
	INJ	5365.8	(90.3)	58.8	(1.0)	240.2	(4.0)	278.7	(4.7)	5943.5^{A}	391.2^{A}
	INJ + DMPP	2572.4	(81.7)	58.8	(1.9)	238.8	(7.6)	278.7	(8.9)	3148.7^{BC}	207.1^{B}
	INJ + DMPSA	3923.5	(87.1)	58.8	(1.3)	242.0	(5.4)	278.7	(6.2)	4503.0^{B}	283.6^{AB}
	INJ + DMPP & DMPSA	2293.9	(79.9)	58.8	(2.0)	238.4	(8.3)	278.7	(9.7)	2869.9^{BC}	183.1^{B}
	INJ + Nitrapyrin	3677.7	(86.4)	58.8	(1.4)	239.1	(5.6)	278.7	(6.6)	4254.3^{B}	280.0^{AB}
	INJ + DCD	2356.4	(80.4)	58.8	(2.0)	238.6	(8.1)	278.7	(9.5)	2932.5^{BC}	198.7^{B}

CON= unfertilized control; INC= slurry incorporation; INJ= slurry injection; for NI abbreviations see Table 4.1; CO_2 eq.= CO_2 equivalents. CO_2 eq. were calculated according to the *IPCC* (2007) guidelines. Before conversion into CO_2 equivalents, annual N_2O emissions from fertilized treatments were not corrected for background emission.

Evaluating the fertilization strategies across both years in terms of their atmospheric burden, NIs (tested in both years) led to a reduction in fertilization-induced GHG release from slurry injection that did not differ significantly from slurry incorporation (Figure 4.3). The combination of DMPP & DMPSA tended to show the highest potential in reducing fertilization-induced GHG release over both years compared to injection of slurry alone.

Using a *t-test* to compare these two treatments (INJ *vs*. INJ + DMPP & DMPSA) separately, the difference was statistically significant (*p=0.028*). A significant difference concerning fertilization-induced CO_2 equivalents across both years was also found between the INJ and INJ + DCD treatment by applying the *t-test* at *p=0.03*7.

4.5 Discussion

4.5.1 Effect of NIs and environmental conditions on N_2O release

4.5.1.1 N_2O fluxes over time

In general, N_2O fluxes were highest in the periods after slurry application due to increased N_{min} contents and higher amounts of easily available C in combination with enhanced soil respiration or soil moisture contents (*Dosch and Gutser*, 1996; *Flessa and Beese*, 2000; *Velthof and Mosquera*, 2011). In agreement with *Velthof and Mosquera* (2011) and *Wulf et al.* (2002b), slurry injection promoted denitrifying conditions more than broadcast application with subsequent incorporation, and resulted in higher N_2O fluxes. The effect of application technique on N_2O release at this study site has been discussed in detail in Chapter 3.5.1. In agreement with *Comfort et al.* (1990), it was assumed that the N_2O fluxes occurring one day after fertilization could be attributed to a reduction in soil-derived NO_3^- due to increased soil moisture through slurry amendment. The lower N_2O peaks immediately after fertilization in the second year with a lower initial N_{min} level reinforced this assumption.

The addition of NIs delayed the NH_4^+ conversion process and resulted in lower N_2O fluxes over a certain period after fertilization (*Dittert et al.*, 2001; *Vallejo et al.*, 2005), in which the duration of the period with inhibition differed between NIs and years.

Generally, in this study, the investigated NIs could be differentiated into two groups: NIs that tended to have their highest inhibitory effect in the early period after application (DMPP, DCD) and those that demonstrated their highest inhibitory effect at a later period (DMPSA, Nitrapyrin, TZ & MP). The combination of DMPP & DMPSA combined the inhibitory effect of both groups.

In comparison to DMPP's inhibition of nitrification immediately after slurry injection (*Dittert et al.*, 2001), the delayed inhibitory effect of DMPSA might be caused by the presence of a succinic group instead of phosphate. Therefore, a microbial degradation

of succinic acid must take place before the active inhibiting substance is released, which then postpones the inhibitory effect. This mechanism has already been confirmed for the combination of DMPSA and mineral ammonium fertilizers (*Guardia et al.*, 2018; *Pacholski et al.*, 2016).

The delayed inhibitory effect of the substituted heterocyclic N compound Nitrapyrin was surprising due to its generally known high volatility and decreasing persistence under warm soil conditions (*Slangen and Kerkhoff*, 1984; *Subbarao et al.*, 2006), which dominated at the start of the vegetation periods (Figure 4.1f). It is conceivable that the new capsule suspension, which should stop the moderate volatility, leads to a higher persistence and delayed release of Nitrapyrin.

During these two-year observations, it was evident that both earlier and later inhibitory NI maximums might offer advantages and disadvantages concerning N_2O mitigation.

In the first year, which was characterized by warm soil conditions and less precipitation in the period after fertilization, heavy rainfall strongly promoted denitrification one month after slurry injection. Therefore, injection treatments containing NIs with delayed inhibition (DMPSA, DMPP & DMPSA, Nitrapyrin, TZ & MP) tended to show a greater potential for reducing N_2O release compared to inhibitors with an already decreasing inhibitory effect (DMPP, DCD).

After fertilization in 2016, cooler soil conditions extended the timeframe of efficient inhibition of DMPP and DCD and postponed the maximum inhibitory effect of DMPSA and Nitrapyrin, probably due to a lower degradation rate. In contrast to 2015, after a rewetting event four weeks after fertilization in 2016 as well, injection treatments containing NIs with an earlier inhibition maximum (DMPP, DMPP & DMPSA, DCD) resulted in a more efficient N_2O reduction than NIs with a delayed inhibitory effect (DMPSA, Nitrapyrin).

4.5.1.2 Annual N_2O emissions

When evaluating the effectiveness of different inhibitory timeframes from the particular NIs concerning N_2O mitigation, a comparison of cumulated annual N_2O emission is useful.

In contrast to the second experimental year, NI addition in the first year did not significantly reduce annual N_2O emissions from slurry injection, despite all NIs tending to reduce N_2O release. The smaller and shorter inhibitory effect in general across all NIs in the first year could probably be attributed to warmer soil conditions in the period after fertilization, resulting in a more rapid degradation of the active compounds. The higher microbial respiration after fertilization in 2015 confirmed this assumption. Moreover, this observation is in agreement with numerous studies that have detected a decreasing persistence of NIs with increasing soil temperatures (*Chen et al.*, 2010; *Irigoyen et al.*,

2003; *Slangen and Kerkhoff*, 1984; *Subbarao et al.*, 2006; *Zerulla et al.*, 2001). While weak inhibitory effects with a low reduction of annual N_2O emissions were observed in the first experimental year, the combination of DMPP & DMPSA tended to have the highest inhibitory efficiency, confirming the assumption that the combination of an earlier (DMPP) and later (DMPSA) inhibition of nitrification covered the longest timeframe with the greatest efficiency in terms of NH_4^+ stabilization.

In the second year, which was characterized by environmental conditions that were more representative for the study site in the period after fertilization (*Agrarmeteorologie Baden-Württemberg*, 2017), the addition of all NIs resulted in a significant reduction of annual N_2O emission when using slurry injection. In that year, slurry injection with NIs was on a comparable level to broadcast slurry incorporation (INC).

Moreover, the addition of DMPP, DMPP & DMPSA and DCD resulted in N_2O emissions that did not differ significantly from the background emission (CON), demonstrating the high efficiency of these active compounds.

A comparable efficiency of the two NIs DMPP and DCD has already been confirmed in previous studies concerning NH_4^+ conservation (*Irigoyen et al.*, 2003) and reduction of N_2O release (*Di and Cameron*, 2012). However, a lower inhibitory effect of DCD under conditions promoting leaching has also repeatedly been reported (*Ruser and Schulz*, 2015; *Zerulla et al.*, 2001), due to it having a water solubility several times greater than NH_4^+ (*Hendrickson and Keeney*, 1979). However, this assumption could not be confirmed in the present study, even with more frequent precipitation in the second year. It is conceivable that the medium heavy soil texture in combination with the smearing effect of the injector tines in the slot zone reduced the impact of downwards-directed water flow (*Vallejo et al.*, 2005).

In the evaluation of the different NIs with regard to the release behavior of their active compounds, NIs with an earlier inhibitory effect after slurry injection tended to be more efficient at reducing N_2O compared to inhibitors with a delayed inhibitory effect. The greater N_2O reduction from NIs with a faster release behavior was probably due to conditions favoring denitrification that mainly dominate during the earlier period after slurry injection. Thus, an early inhibition of NH_4^+ oxidation is of particular importance when using slurry injection to desynchronize NO_3^- and C availability. However, as shown in the first year, under warmer soil conditions a delayed inhibitory effect can also be beneficial. Whether the performance of a higher inhibitory effect in the earlier or later period after slurry injection is an advantage or a disadvantage obviously greatly depends on the environmental conditions (temperature and precipitation).

On the basis of this, the combination of DMPP & DMPSA seemed to be the most efficient NI at reducing annual N_2O emissions in both years due to efficient inhibition in both the earlier and later periods after slurry injection.

Consequently, a combination of inhibitors with differing release behaviors (such as DMPP & DMPSA) proved to be the most advisable way of efficiently reducing N_2O release due to a lower dependence on environmental conditions (*e.g.* temperature, precipitation) in the period after fertilization.

However, all NIs investigated in both years together with slurry injection reduced fertilization-induced N_2O emissions over both years to a level comparable with slurry incorporation. Thus, in addition to slurry incorporation, slurry injection combined with NI application also proved to be an appropriate fertilization strategy regarding N_2O release under the given conditions.

The NI addition to broad slurry incorporation (INC + DMPP & DMPSA) also tended to decrease N_2O emissions, but due to the generally lower denitrification rate of this application technique (INC) compared to injection (*Dosch and Gutser,* 1996; *Thompson et al.*, 1987), the potential of reducing N_2O emission from the INC treatment remained lower.

4.5.1.3 Emission factors

The N_2O reduction potential of the differing NIs was reflected by their N_2O EFs, which were reduced by a third in comparison to the application of slurry alone (mean across all treatments and years). The strong and significant reduction in N_2O emissions through the addition of DMPP, DMPP & DMPSA or DCD together with injection and/or incorporation resulted in EFs close to or below the *IPCC* (2006) default value of 0.01 kg N_2O-N (kg N)$^{-1}$. Thus the enzymatic inhibition of NH_4^+ oxidation could be confirmed as an appropriate approach for mitigating N_2O release, especially for slurry injection.

However, the present results as well as results from previous studies (*Bouwman et al.*, 2002; *Vallejo et al.,* 2005; *Van Nguyen et al.*, 2017; *Velthof and Mosquera,* 2011) show that the N_2O EF for slurry application has not been quantified appropriately and illustrate the relevance of considering the use of NIs as well as application technique and environmental conditions to enable a more accurate calculation of N_2O releases. Consequently, an adaption of the current IPCC methodology is desirable.

4.5.2 Impact of NIs regarding N removal and yield

Previous studies have noted an improved yield and/or NUE through the addition of DMPP, DCD (*Fangmeier et al.*, 1994), Nitrapyrin (*McCormick et al.*, 1984; *Sutton et al.*, 1986) and TZ & MP (*Federolf et al.*, 2016) to liquid manure due to reduced N losses. However *McCormick et al.* (1984) and *Schmitt et al.* (1995) have reported a variable

yield response of maize plants after manure injection with NIs. The authors have attributed the non-beneficial impact of NIs on yield response and N offtake to high levels of plant-available N in soil or a high N application rate and environmental conditions unfavorable for N losses. In the present study, the addition of NIs did not increase either the DM yield or N removal from slurry injection treatments. Even an increased N-fertilization rate by 50 % from the INJ treatment (data not shown) did not significantly increase the yield response or N removal in either year. Thus, in agreement with the results of *McCormick et al.* (1984), an already exceeded optimal N dose was assumed. This meant that soil N was not the limiting factor for yield and N offtake and therefore an additional and prolonged N supply through NIs did not lead to any improvement. Furthermore, it could be assumed that the risk of NO_3^- leaching losses on the medium-textured soil from the study site was low during the vegetation period and thus the beneficial effect of NIs through NH_4^+ conservation also remained small (*Gaines and Gaines*, 1994). In agreement with this assumption, a shorter NH_4^+ stabilization of the NI DCD after rainfall events due to its high water solubility did not influence yield or N removal, as also reported by *Fangueiro et al.* (2009) on a coarse-textured soil.

Only the broadcast incorporation treatment with the combination of DMPP & DMPSA was inferior to all injection treatments. A lower yield and N removal of slurry incorporation in comparison to injection have been reported in several studies (*Federolf et al.*, 2016; *Schmitt et al.*, 1995; *Sutton et al.*, 1982) and mainly explained by the nutrient distribution to maize roots being less concentrated and a higher N immobilization rate (*Sørensen and Amato*, 2002). Comparing slurry incorporation with and without NI, the NI addition tended to decrease N removal and yield response. It could be assumed that the prolonged NH_4^+ stabilization resulted in decreased plant availability of N, especially under dry soil conditions at the start of the vegetation period (*Mattila*, 2006). This putative N deficiency from maize plants in the early growth stage (INC + DMPP & DMPSA treatment) was probably not resolved until harvest.

4.5.3 Atmospheric burden

As it was already confirmed in Chapter 3, slurry injection without NIs is not an appropriate fertilization strategy for livestock farmers in this experimental region owing to excessively high N_2O emissions. Due to the high proportion of N_2O in total GHG release (between 64 % and 93 %), the reduction of this trace gas has proved to be of particular importance for reducing the atmospheric burden. In the present study, NIs reduced annual N_2O emissions from slurry injection by 36 % (mean across all treatments and years), confirming the assumption of *Smeets et al.* (2009) of an improved GHG balance through NIs by reducing N_2O release by one third. The NI-induced reduction in total

GHG emissions from slurry injection was 24 % and 33 % in the first and second experimental year respectively (mean across all treatments). Combining the limited reduction of the atmospheric burden from slurry injection through NI addition in the first year and the strong reduction in the second year, NIs achieved a decrease in the mean fertilization-induced CO_2 equivalents from both years that was comparable to slurry incorporation.

Therefore, NIs with a greater inhibitory effect immediately after slurry injection (DMPP, DCD) tended to be more efficient at reducing GHG release compared to NIs with a delayed maximum inhibitory effect (DMPSA, Nitrapyrin). A combination of two active compounds, one with an earlier and the other with a delayed inhibitory maximum (DMPP & DMPSA), tended to exceed all the remaining NIs investigated with regard to the reduction of total and yield-related GHG release.

Consequently, slurry injection proved to be an appropriate fertilization strategy for livestock farmers in this experimental region, but only if N_2O emissions are reduced through the use of NIs. Therefore, the combination of DMPP & DMPSA turned out to be the most appropriate additive, followed by DCD and DMPP, whereas DMPSA alone and Nitrapyrin were less advisable due to their lower performance in reducing the GHG release in both years on average.

The tendency for reduced GHG release when NI was added to slurry incorporation indicated the potential for an even greater improvement from this application technique concerning the atmospheric burden by inhibiting nitrification. However, due to the tendency for decreased N removal and yield in particular, further research is necessary to evaluate the use of NIs combined with slurry incorporation.

Further research is also needed to measure NH_3 emissions after slurry incorporation or injection with and without NIs. Due to the indirect N_2O emission associated with NH_3, losses from treatments with NIs could be higher than the assumed values in the present study, which were based on measurements from the INJ or INC treatment without NIs. Increased NH_3 losses have repeatedly been reported through the use of NIs together with surface placement of NH_4^+-based fertilizers (*Kim et al.*, 2012; *Soares et al.*, 2012), mainly caused by prolonged NH_4^+ availability, which may increase NH_3 losses (*Asing et al.*, 2008; *Kim et al.*, 2012; *Zaman et al.*, 2009). Due to the absence of data on NH_3 emissions affected by NIs together with slurry injection or incorporation, the calculation had to be simplified here. However it may be hypothesized that NH_3 losses from treatments with NIs do not substantially exceed NH_3 emissions from measured losses without NI due to the greatly reduced contact with the above soil atmosphere (*Menéndez et al.*, 2006; *Webb et al.*, 2010).

4.6 Conclusions

In the present study, in addition to broadcast incorporation of organic fertilizers (Chapter 3), slurry injection also proved to be an appropriate fertilization strategy regarding N_2O release and thus the atmospheric burden when combined with the use of NIs and in climate conditions typical for the experimental region (not promoting NI degradation by high soil temperatures).

Moreover, slurry injection has a lower risk of NH_3 losses (Chapter 3; *Wulf et al.*, 2002a), requires less manpower (*KTBL*, 2009) and tends to have higher N removal as well as increased yields when compared to harrowing after surface application. Hence, this fertilization strategy protects the earth's climate by efficiently reducing N_2O and NH_3 losses and guarantees beneficial effects for farmers by avoiding extra costs for N-fertilizers (*Cassman et al.*, 2002) and reducing working time (*KTBL*, 2009).

Under the given conditions, NIs that reduced denitrifying conditions during the main emitting period immediately after slurry injection (DCD, DMPP) tended to have a greater potential for reducing the atmospheric burden than NIs with a prolonged inhibitory effect due to the delayed release behavior of the active compounds (DMPSA, Nitrapyrin) that inhibit nitrification. The combination of the two active compounds DMPP & DMPSA tended to be the most appropriate additive for reducing GHG release after slurry injection in both years, probably due to it covering a longer inhibitory period due to having one active compound with immediate (DMPP) and one with delayed (DMPSA) release of DMP.

Nevertheless, further long-term studies (measuring N_2O emissions over an entire year) are necessary to test various marketable NIs simultaneously in order to evaluate each inhibitor's efficiency under varying environmental conditions and management factors. On the basis of such a comprehensive dataset, an even more optimized use of NIs regarding the atmospheric burden might be realized through inhibitor application with greater site-specific and management-specific efficiency at reducing N_2O emissions.

Moreover, a further reduction in GHG release from silage maize production is conceivable through an additional NI application before fertilization. Thus NO_3^- availability could probably be diminished immediately after slurry injection, preventing N_2O formation from soil-derived N under conditions favoring denitrification.

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5 Effect of cattle slurry application and nitrification inhibitors on CH_4 dynamics

5.1 Abstract

Methane (CH_4) is a climate relevant trace gas which contributes substantially to greenhouse effect. Soils play an important role in global CH_4 cycle and can function as sink or source for atmospheric CH_4. Hence, aeration is the key factor which determines CH_4 dynamics in soils. Generally, well-aerated arable soils are known to serve as CH_4 sink due to methanotrophic bacteria oxidizing CH_4 to carbon dioxide (CO_2). However, in anaerobic soil zones, conditions for CH_4 formation by methanogens are favorable. Depending on which is the dominant process in soil, the soil is a net CH_4 sink or source. To identify management factors influencing CH_4 dynamics in soils with the aim to decrease the atmospheric CH_4 concentration, this study investigated how CH_4 consumption or release are affected by different nitrogen (N)-fertilizer forms, different application techniques of cattle slurry and by the addition of different nitrification inhibitors (NIs). Therefore, CH_4 fluxes from an unfertilized control (CON), from a treatment with mineral N fertilizer (MIN), with banded surface application and broadcast incorporation of cattle slurry (INC), and with cattle slurry injection (INJ) were investigated during silage maize production on a Haplic Luvisol in southwest Germany. Treatments INC and INJ were also combined with NIs (INC&NI, INJ&NI). Except for the INC + NI treatments (exclusively investigated in the second year) and one of the six INJ + NI (exclusively investigated in the first year), all remaining treatments were investigated during two years with frequent CH_4 measurements. In both years, soils in the CON, MIN and INC treatments were net CH_4 sinks with an uptake of 606, 116, and 862 g CH_4-C ha^{-1} yr^{-1} in the first and 314, 137, and 723 g CH_4-C ha^{-1} yr^{-1} in the second year, respectively. In contrast slurry injection promoted methanogenesis in the periods after injection resulting in a net CH_4 source which was significantly different from slurry incorporation in the first and in the second year with net emissions of 5645 and 643 g CH_4-C ha^{-1} yr^{-1}, respectively. The addition of NIs reduced the annual CH_4 emissions from soils in the INJ + NI treatments (in mean by 52 % and 43 % in the first and second year, respectively) and increased CH_4 uptake by soils in the INC + NI treatments by 20 %. Since all NIs induced a CH_4 reduction either by reduced CH_4 emissions or increased CH_4 uptake, this general effect of NIs may be due to an increase of O_2 availability in soil by the inhibition of nitrification. Comparing CH_4 dynamics of both years, CH_4 uptakes and CH_4 emissions were generally higher in the warmer and dryer first year than in the second year. In the second year, O_2 depletion induced by higher soil moisture was probably the main reason for limiting CH_4 production and CH_4 oxidation. Moreover, under wetter soil conditions in the second year, enhanced aeration through additional

harrowing in the INC treatments tended to increase CH_4 oxidation. Consequently, the use of NIs as well as an enhanced aeration through additional harrowing might be a promising tool to improve CH_4 budgets in silage maize production.

5.2 Introduction

CH_4 is a climate relevant trace gas which contributes 16 % to greenhouse effect (*IPCC*, 2014b). Despite the short atmospheric residence time of approximately 10 years, this hydrocarbon has a global warming potential (GWP) 23 times higher than the equivalent amount of CO_2, mainly caused by its ability to absorb infrared radiation (*European Commission*, 2010; *Le Mer and Roger*, 2001). However, the potency of its GWP has recently been disputed due to interactions between CH_4 and aerosols which have not yet been taken into account and which would raise the relative GWP of CH_4 to about 33 (*Sanderson*, 2009). This uncertainty indicates that CH_4 might be an even more potent greenhouse gas than previously estimated and it demonstrates the importance of reducing the release of this trace gas into the atmosphere.

Soils with a low aeration such as natural wetland soils or soils from paddy rice cultivation, and landfill soils are the major contributor to annual global CH_4 production with a share of more than 50 % (*Denman et al.*, 2007). In contrast, well-aerated soils are known to serve as CH_4 sink through CH_4 oxidation and account for nearly 5 % of the annual global CH_4 sink (*Denman et al.*, 2007; *Topp and Pattey*, 1997). Thus, soils can function as sink or source for atmospheric CH_4 and they have an important function in CH_4 cycle. Consequently, the decrease of CH_4 emission and the increase of CH_4 uptake from soils are appropriate tools towards lowering atmospheric CH_4 concentrations. Thereby, aeration is the key factor which determines whether CH_4 production or consumption dominate in soils (*Le Mer and Roger*, 2001).

Methanogenesis, the process of CH_4 formation completes the last step in an anaerobic fermentation process of organic matter (OM) by microorganisms for which soil redox potentials of −150 mV or less are required (*Chidthaisong and Conrad*, 2000; *Masscheleyn et al.*, 1993; *Yagi et al.*, 1994). During this transformation process, microorganisms mineralize OM completely according to the reaction : $C_6H_{12}O_6 \rightarrow 3\ CO_2 + 3\ CH_4$ (*Le Mer and Roger*, 2001) whereby CH_4 emission rates increase with decreasing redox potential (*Kludze et al.*, 1993; *Masscheleyn et al.*, 1993).

In contrast, for the aerobic process of CH_4 uptake, O_2 and CH_4 availability is essentially required for oxidizing CH_4 to CO_2 by methanotrophic bacteria (methanotrophs) (*Hanson et al.*, 1996). Thus, CH_4 oxidation might be decreased by reduced air diffusion into the soil (*Striegl*, 1993) or is also known to be mitigated by N-fertilizer application (*Hütsch et al.*, 1993).

Whether an environment functions as net CH_4 sink or source depends on the balance between these two processes (CH_4 consumption by methanotrophic and CH_4 production by methanogenic microorganisms). Environmental conditions (*e.g.* temperature, soil redox potential, soil moisture, soil type) or management factors, such as N-fertilization measures, influence the balance between these CH_4 transforming processes strongly (*Topp and Pattey*, 1997).

In previous studies it was confirmed repeatedly that the application, especially of N-fertilizers rich in ammonium (NH_4^+), such as slurry or calcium ammonium nitrate (CAN) reduces CH_4 oxidation through an enzymatic inhibitory effect (*Bronson and Mosier*, 1994; *Conrad and Rothfuss*, 1991; *Dunfield and Knowles*, 1995; *Hansen et al.*, 1993). Firstly, this inhibitory effect can be explained by competitive inhibition of methane monooxygenase (MMO) through NH_4^+ which competes with CH_4 for binding site (*Bédard and Knowles*, 1989). Secondly, the increased concentration of nitrite (NO_2^-) as end product from methanotrophic nitrification which was induced by NH_4^+ fertilized, has a toxic effect on the organisms, probably due to inhibiting the formate dehydrogenase and thus the last step of CH_4 oxidation (*Jollie and Lipscomb*, 1991; *Schnell and King*, 1994).

Beside the enzymatic inhibition of N-fertilizers, it was frequently shown that the method of fertilizer application might also have an impact on CH_4 dynamics in generally well-aerated arable soils. On the one hand, application technique-induced changes in soil physical properties might influence CH_4 oxidation by increasing (*e.g.* enhanced bulk density through harrowing) or decreasing (*e.g.* blocked soil pores through the smearing effect of injector tines) CH_4 and O_2 diffusion into soil (*Hansen et al.*, 1993; *Ruser et al.*, 1998; *Wulf et al.*, 2002b). On the other hand, the soil redox potential can also be affected by application technique through differing fertilizer placement which may result in soil compartments with anoxic conditions increasing CH_4 production or reducing CH_4 oxidation. Anoxic redox potentials can be induced by fertilizers with high water content or which increase microbial respiration, such as slurry or digestates (*Dosch and Gutser*, 1996; *Flessa and Beese*, 2000; *Wulf et al.*, 2002b).

Concentrated slurry placement through injection into the soil was repeatedly reported as creating soil conditions favoring methanogenesis (*Flessa and Beese*, 2000; *van den Pol-van Dasselaar et al.*, 1999; *Wulf et al.*, 2002). *Flessa and Beese* (2000) detected redox potentials comparable with those of submerged soils from paddy rice cultivation in an injection slot after cattle slurry application into loamy soil. Slurry injection reduced the redox potential to nearly −200 mV and resulted in net CH_4 release. Similarly, *Wulf et al.* (2002) reported net CH_4 emissions after cattle slurry amendment with varying application techniques, where slurry injection showed by far the highest and the most prolonged

positive CH_4 fluxes. In contrast to concentrated slurry placement by injector, in numerous studies, positive CH_4 fluxes in the short-term, immediately after broadcast application, were rather attributed to volatilization of dissolved slurry CH_4 produced during storage than to net CH_4 production in soil (*Chadwick et al.*, 2000; *Sommer et al.*, 1996; *van den Pol-van Dasselaar et al.*, 1999; *Wulf et al.*, 2002).

Querying this claim, *Van Nguyen et al.* (2017) detected reduced O_2 availability over 18 days after broad cattle slurry application on the soil surface. They did not determine CH_4 fluxes, however conditions favorable for NO_3^- reduction were observed simultaneously to O_2 depletion. Furthermore, the authors determined a much lower O_2 consumption when the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) was added to the slurry. Thus, they concluded that the transformation of NH_4^+ by nitrifiers can influence O_2 depletion substantially in the period after slurry amendment. Hence, it is quite conceivable that a reduced O_2 consumption in the period after slurry application through NI addition might result in decreased CH_4 emission or increased CH_4 oxidation. Results from *Weiske et al.* (2001) who tested the NIs DMPP and dicyandiamide (DCD) in combination with a mineral N-fertilizer confirmed this suggestion. Although neither NIs affected CH_4 fluxes significantly, DMPP tended to increase the CH_4 uptake.

Up to now, little is known about the impact of NI addition concerning regulating CH_4 fluxes. As a result, and due to a lack of full annual CH_4 measurements on arable soils, the present study estimated the impact of fertilizer form (mineral *vs.* organic fertilizer), cattle slurry application techniques (broadcast incorporation *vs.* injection) as well as the use of NIs together with cattle slurry on CH_4 fluxes over two experimental years. The aim was to verify the following hypotheses: (i) CH_4 consumption of soils after broadcast slurry incorporation is higher than after broadcast mineral fertilization, (ii) cattle slurry injection increases CH_4 fluxes (emission) compared to a broadcast slurry incorporation (consumption), (iii) NIs reduce CH_4 emissions attributed to methanogenesis or increased CH_4 oxidation compared to sole slurry application.

5.3 Material and methods

5.3.1 Study site, crop management and treatments investigated

The experiment was conducted on "Heidfeldhof" which is one of the experimental stations of the University of Hohenheim near Stuttgart (48°43'0.30'' N; 9°11'30.64'' E; 404 m a.s.l.). Soil type are classified as Haplic Luvisol and soil texture consists of 2 % sand, 74 % silt, and 24 % clay. The study site is characterized by a mean annual precipitation of 691 mm and an annual mean air temperature of 10.1 °C (*Agrarmeteorologie Baden-Württemberg*, 2017). The top soil (0-30 cm depth) had an initial pH of 7.0 (0.01 *M* $CaCl_2$), a C_{org} content of 0.72 % and a N_t content of 0.10 %. At the beginning of the

experimental period, the initial mineral N (N_{min}) content (0-60 cm soil depth) was 44 and 21 kg N ha^{-1} in the first and second year, respectively.

We established a completely randomized block design with four replicates in the first year (28 April 2015 – 19 April 2016) and in the second experimental year (28 April 2016 and 3 May 2017). The study site of the second year was on an adjacent field from that of the first year. Plot size was 10 m x 3 m.

The investigated treatments differed in N fertilization or application technique: an unfertilized control (CON), a broadcast mineral fertilization with calcium ammonium nitrate (MIN), a banded cattle slurry application on the soil surface with subsequent broadcast incorporation (INC) and a cattle slurry injection (INJ). To quantify the effect of NI addition on CH_4 fluxes, several NIs, listed in Table 5.1, were investigated in the INC and INJ treatments. Except for the treatment INJ + TZ & MP (tested only in the first experimental year) and the treatment INC + DMPP & DMPSA (tested only in the second experimental year), all remaining treatments were tested in both experimental years. NI amounts added to the slurry were identical as those described in Chapter 4 using for treatments with DMPP a quantity of 6 L ha^{-1} from the liquid formula ENTEC® FL (containing 25-50 % ammonium nitrate and 1-5 % of the active compound DMPP) (*EuroChem Agro*, 2014). For DMPSA, the equivalent amount of DMP as in the DMPP treatment was used. For the treatments with the combination of the two active compounds DMPP and DMPSA, half of the above mentioned amounts of each NI, were added to the slurry. Nitrapyrin as N-LOCK™ Nitrogen Stabilizer was tested in the present study with the amount of 2.5 L ha^{-1} (*The Dow Chemical Company*, 2012). For the treatment with the solution of the two pyrazole derivatives (containing 3.00-3.25 % TZ; 1.50-1.65 % MP), with the trade name PIADIN®, a quantity of 6 L ha^{-1} was applied (*SKW Stickstoffwerke Pisteritz GmbH*, 2007). DCD was added to the slurry in pure form using 102.3 g DCD m^{-3} $slurry^{-1}$ equivalent to the 12.5 times amount of DMPP.

For the INC treatments, slurry was incorporated by a circular harrow with a working depth of 15 cm, 2 h after drag hose application. The injecting cultivator placed the slurry in 15 cm soil depth with a share width of 5 cm. The space between trail hoses or injector tines was 75 cm. In both years, maize (*Zea mays* L., variety "Amadeo") was sown with a plant density of 12,700 plants ha^{-1}. Maize rows were placed 5 cm next to the injection slot and with a sowing depth of 5 cm. In 2015, maize was sown on 13 May and harvested on 26 August as silage maize.

Table 5.1: Abbreviation and description of the different treatments with N-fertilizer form, application technique, nitrification inhibitor added (active substance(s) and trade name) and sampling period.

	Treatment		N-fertilizer	Application technique	Nitrification inhibitor	Trade name	Sampling period	
							1st yr	2nd yr
i	**CON**		unfertilized	-	-	-	X	X
ii	**MIN**		CAN[1]	broadcast	-	-	X	X
iii	**INC**[2]		cattle slurry	trail hose + incorporation	-	-	X	X
iv	**INC**	**+ DMPP & DMPSA**	cattle slurry	trail hose + incorporation	3,4-dimethylpyrazole phosphate 3,4-dimethylpyrazole succinic acid	ENTEC® FL currently not traded		X
v	**INJ**[3]		cattle slurry	injection	-	-	X	X
vi	**INJ**	**+ DMPP**	cattle slurry	injection	3,4-dimethylpyrazole phosphate	ENTEC® FL	X	X
vii	**INJ**	**+ DMPSA**	cattle slurry	injection	3,4-dimethylpyrazole succinic acid	currently not traded	X	X
viii	**INJ**	**+ DMPP & DMPSA**	cattle slurry	injection	3,4-dimethylpyrazole phosphate 3,4-dimethylpyrazole succinic acid	ENTEC® FL currently not traded	X	X
ix	**INJ**	**+ Nitrapyrin**	cattle slurry	injection	nitrapyrin	N-LOCK™ Nitrogen Stabilizer	X	X
x	**INJ**	**+ DCD**	cattle slurry	injection	dicyandiamide	Alzon®	X	X
xi	**INJ**	**+ TZ & MP**	cattle slurry	injection	1,2,4 triazole & 3-methylpyrazole	PIADIN®	X	

[1]calcium ammonium nitrate, [2]incorporation, [3]injection

In 2016, maize sowing was done on 4 May and harvest was conducted on 13 September. Seedbed preparation was performed one week before sowing with a cultivator in both years. After maize chopping, maize stubbles were mulched and subsequently incorporated with a cultivator. After the maize vegetation periods, the field lay fallow without any further soil cultivation activity and crop management practice.

In the first year, N-fertilization took place twice on 10 May (140 kg N ha^{-1}) and 18 June (30 kg N ha^{-1}). In order to avoid damage to growing maize plants due to agglomeration of the loamy soil, the second fertilization of the slurry treatments was surface-applied with a trail hose instead of injection or incorporation without NI additions. Due to a higher total N concentration of the cattle slurry in the second year, N-fertilization took place only once (3 May).

Corresponding to the maximum amount of organic N-fertilizer permitted by the German legislation on N-fertilization (*DüV*, 2006), in the first year, 170 kg N ha^{-1} (based on total N) was applied in all fertilized treatments. Due to lower initial N_{min} content in soil in the second year, we applied 190 kg total N ha^{-1} to ensure a better compatibility between the two study years.

In both years, the amount of total N applied was equal for all fertilized treatments. As a consequence, the mineral fertilized treatment contained twice as high an amount of inorganic N applied (100 % inorganic N, containing 50 % NH_4^+ and 50 % NO_3^-) than the organically fertilized treatments (approximately 50 % of total N applied as NH_4^+). Hence, the amount of NH_4^+ fertilized was equivalent for mineral and organically fertilized treatments.

5.3.2 CH_4 fluxes: sampling, laboratory analyses and calculation

Over both experimental years, CH_4 flux measurements were conducted weekly and event-based with the closed chamber method (*Hutchinson and Mosier*, 1981). Sampling procedure was conducted as described in Chapter 3 placing the base frames of the gas chambers area-representative for the particular treatment. Thus, for the INJ treatments, a percentage of 7 % covered the slot zone and 93 % covered the area next to the slot (Chapter 10.2; *Gassner*, 2017). Chamber design and sampling procedure were outlined in depth by *Pfab et al.* (2011). The chromatographically determination of CH_4 concentration in gas samples was determined with a gas chromatograph (GC 450 Greenhouse Gas Analyzer, Bruker Daltonic, Bremen, Germany) which was connected to an autosampler (Gilson GX 281, Middleton, USA). CH_4 concentrations were quantified with a flame ionization detector (FID). For the chromatograms' integration we used Bruker Compass CDS™ 2012 software. CH_4 flux rates were calculated using the package "gasfluxes" (*Fuss and Asger*, 2014) for R software (*R Core Team*, 2016), which selects

the most suitable model for calculating trace gas fluxes (more detailed information is given by *Ruser et al.* (2017)).

5.3.3 Weather data

Measured precipitation and air temperature data were provided by the University's meteorological station, which is located approximately 500 m next to the experimental site. Soil temperature was recorded in soil depths of 5, 10, 15 cm in each of the four replicated blocks separately using data loggers (Logtag, TRIX-8, CIK solutions, Karlsruhe, Germany).

5.3.4 Soil sampling and laboratory analyses

To determine soil moisture and N_{min} contents (0.5 *M* K_2SO_4), soil sampling (0-30 cm) was conducted simultaneously to gas sampling. Soil sample collection was carried out as described in Chapter 3.3.5. Three samples were taken randomly out of each plot with an auger. These soil samples were then pooled from the four replicated plots of each treatment and stored frozen before laboratory analyses. Except for the INJ treatments, samples were taken directly over the injection slot and in the area between the slots, separately.

To determine N_{min} contents of soil samples, 80 mL of 0.5 *M* K_2SO_4 solution were used for extraction with 20 g fresh soil. Extracts were measured using a flow injection analyses (3 QUAAtro.AQ2.AACE, SEAL Analytical, UK). Additionally, soil samples were taken before sowing from each plot separately (0-30 cm, 30-60 cm, and 60-90 cm) to identify the initial N_{min} contents. For determination of gravimetric soil moisture, soil samples were dried at 105 °C for 24 h.

5.3.5 Statistical analyses

Statistical analyses were carried out using the Statistical Software package SigmaStat 3.5. Data were tested for variance homogeneity. Normal distribution of residuals was tested with the Shapiro Wilk Test. The data showed a variance homogeneity without any transformation. A two factorial variance analysis (ANOVA) was done with the factors block and treatment, to test the block effect in each experimental year.

Owing to differences between the two experimental years (*e.g.* precipitation and temperature in the period after fertilization, slurry characteristics, abundance of fertilization, change of experimental site), a one factorial ANOVA was performed separately for each experimental year, to detect differences between the treatments under the given conditions. Significant differences were determined using a pairwise multiple comparison procedure (*Student-Newman-Keuls, $p<0.05$, $n=4$*). The data are presented as arithmetic means with standard errors.

5.4 Results

5.4.1 Weather conditions

In both years, the mean annual air temperature was in line with the long-term annual mean. The annual precipitation in the first and second year was 150 mm and 50 mm below the long-term mean for precipitation. Especially in the period after fertilization in the first year (May up to July) precipitation was 33 % lower than expected. During this period after fertilization in the second year, precipitation was only 11 % less due to a more representative occurrence of rainfall events for this study site.

Within the first 33 days after fertilization, soil temperature in the first year (at a depth of 10 cm) was 3 °C higher compared to the second year (Figure 5.1e).

5.4.2 Temporal pattern of CH_4 fluxes and mineral N

CH_4 fluxes showed a high temporal and spatial variability over all treatments and experimental years (Figure 5.1a, Figure 5.2a, Figure 5.3a). Broadcast mineral (MIN) and organic (INC and INC + DMPP & DMPSA) fertilizer application did not affect the course of CH_4 fluxes noticeably which was indicated by a fairly similar course of CH_4 flux rates as observed in the unfertilized control treatment in both years (Figure 5.1a). During the whole observation period, the unfertilized control treatment varied between CH_4 consumption rates of −68.8 mg CH_4-C m^{-2} h^{-1} up to net CH_4 production of 40.4 mg CH_4-C m^{-2} h^{-1} whereby no relationship to soil moisture, microbial respiration or NH_4^+ content could be observed. Also in the period after fertilization, the fluctuations of CH_4 fluxes from broadcast fertilized treatments (MIN, INC, INC + DMPP & DMPSA) were not significantly correlated with any of the driving variables.

In contrast, CH_4 dynamics from treatments with concentrated slurry placement into the soil by injector were strongly affected by the fertilization measure. Injection treatments showed positive CH_4 fluxes for 26 and 7 days after injection in the first and second year, respectively (Figure 5.2a, Figure 5.3a) where the sole INJ treatment without NI had the highest CH_4 fluxes over the whole observation with flux rates up to 1815 mg CH_4-C m^{-2} h^{-1} (5 days after fertilization, first year). Since NIs were added to the slurry before injection, the positive CH_4 fluxes generally tended to be on a lower level whereas no differences between NIs could be observed.

The course of NO_3^- concentrations seemed also to be affected by slurry application technique. In contrast to broadcast slurry incorporation where NO_3^- concentrations increased more promptly after fertilization (Figure 5.1c), the increase after injection was delayed (Figure 5.2c, Figure 5.3c).

After slurry injection with and without NIs in the first year, NO_3^- concentrations declined for 5 days after fertilization, resulting in a negative correlation between CH_4 fluxes and NO_3^- concentrations for all INJ treatments (r^2=0.47, Pearson Product Moment Correlation, $p<0.05$).

At the point at which net CH_4 production switched to net CH_4 consumption, in both years, NO_3^- concentrations from INJ treatments increased discernibly (Figure 5.2a and c, Figure 5.3a and c). In the periods after fertilization, NH_4^+ stabilization by NIs tended to result in generally lower NO_3^- concentrations (Figure 5.1b and c, Figure 5.2b and c, Figure 5.3b and c).

After the fertilization-induced increase of CH_4 fluxes from INJ treatments, CH_4 fluxes were on a similar low level for the remaining observation periods with fluctuations between −178 mg CH_4-C m^{-2} h^{-1} (INC, 155 days after fertilization in 2016) and 100 mg CH_4-C m^{-2} h^{-1} (INJ, 166 days after fertilization in 2015). Despite increased NH_4^+ concentrations after additional surface fertilization in 2015 (40 days after first fertilization), CH_4 dynamics were not affected by this fertilization measure. Silage maize harvest or maize stubble incorporation also had no effect on CH_4 fluxes.

Comparing the two experimental years concerning CH_4 transformation processes, CH_4 emissions from methanogenesis as well as CH_4 uptakes from CH_4 oxidation were generally higher in the first year which was characterized by warmer and dryer soil conditions, especially in the period after fertilization (Figure 5.1f).

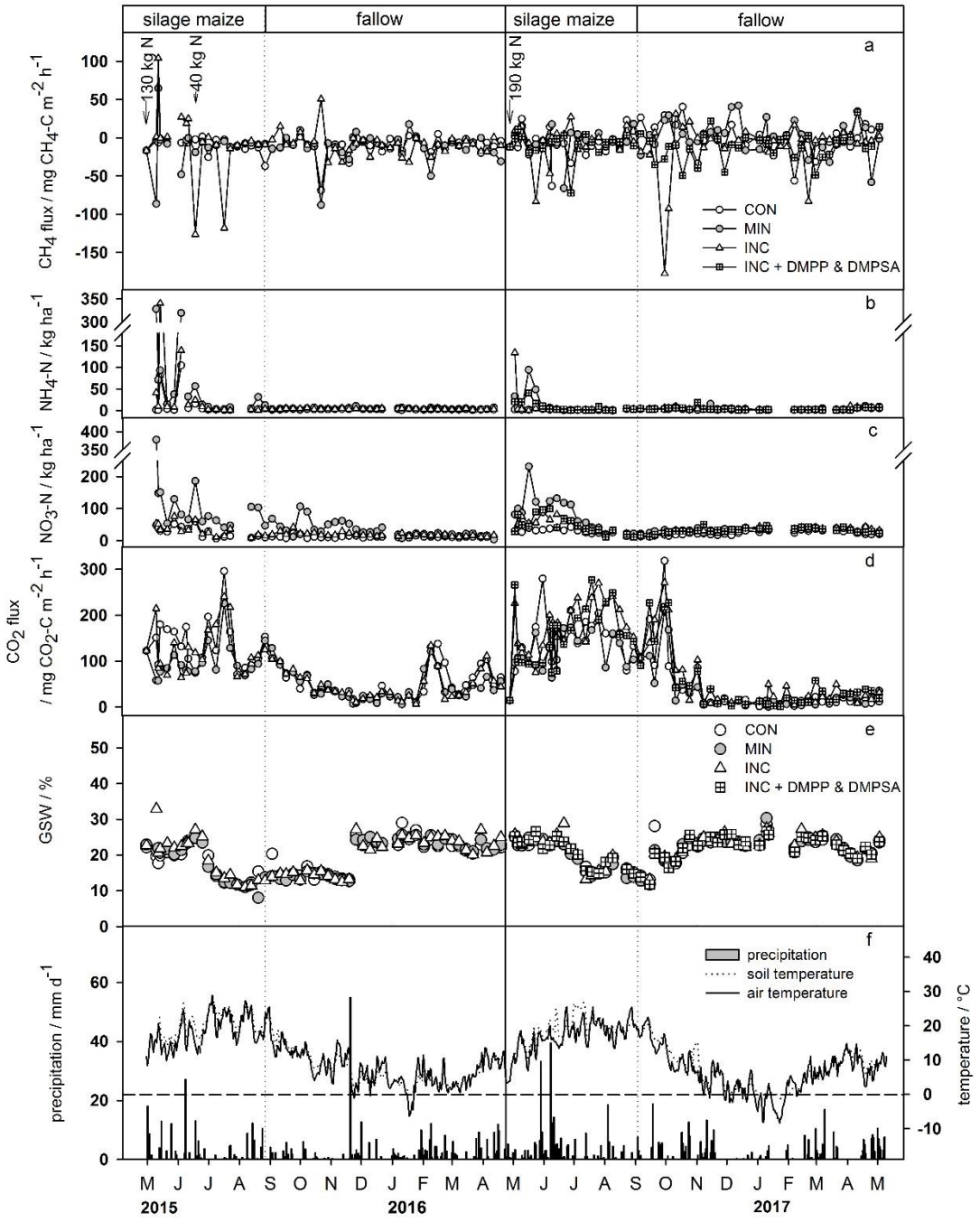

Figure 5.1: Mean CH_4 flux rates (n=4) (a), NH_4^+-N contents (0-30 cm) (b), NO_3^--N contents (0-30 cm) (c), mean CO_2 flux rates (n=4) (d) and gravimetric soil water content (GSW, 0-30 cm) (e) in the unfertilized control treatment (CON), the mineral N-fertilization treatment (MIN) and the broadcast slurry incorporation treatment without (INC) and with nitrification inhibitor (INC + DMPP & DMPSA) over the two experimental years. For nitrification inhibitor abbreviations see Table 5.1. Arrows indicate fertilization measures in the MIN, INC and INC + DMPP & DMPSA treatment. Mean air temperature (solid line), soil temperature at 10 cm depth (dotted line), and daily precipitation (black bars) (f). Error bars are omitted for the purposes of clarity. Data from NH_4^+-N contents, NO_3^--N contents, CO_2 flux rates and gravimetric soil water content are taken from Chapter 3 and 4.

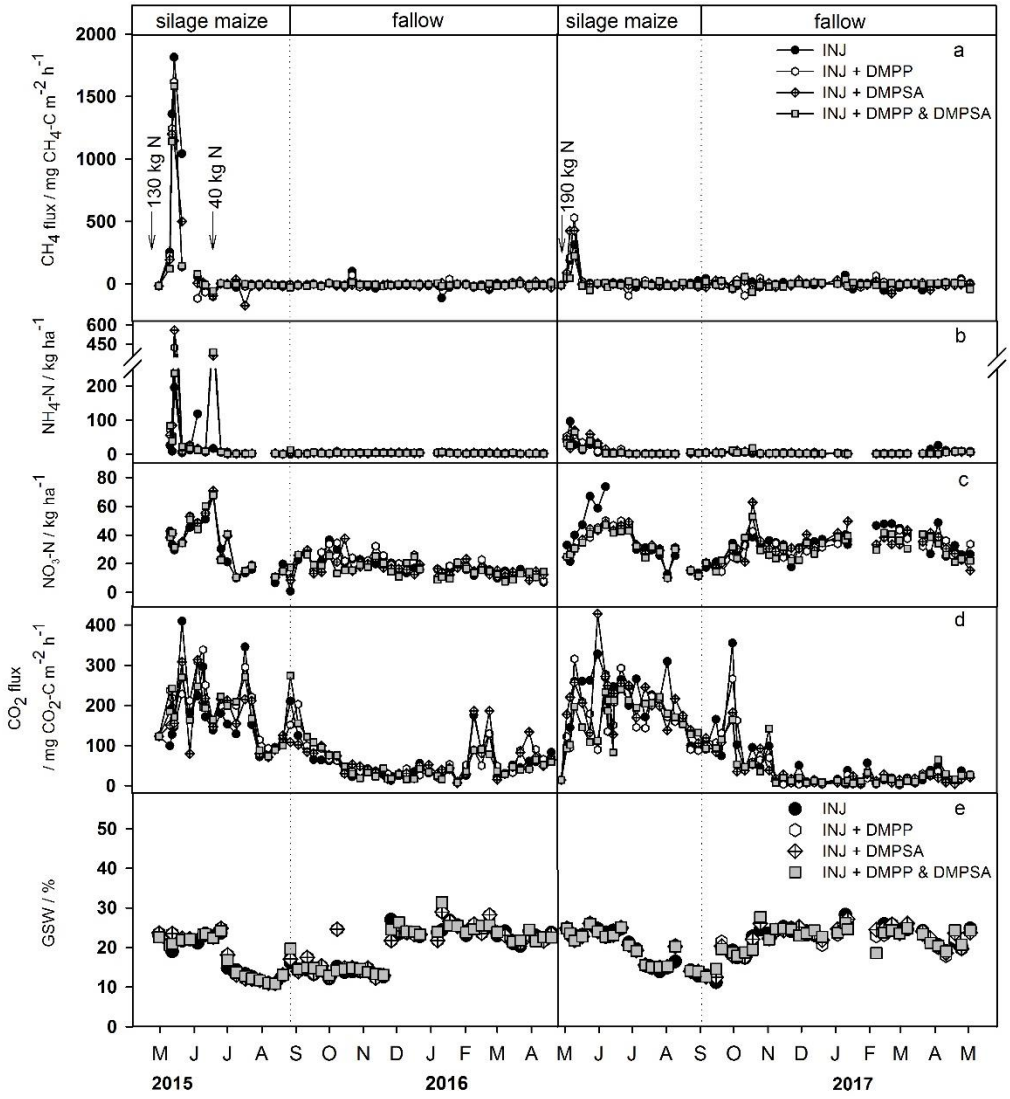

Figure 5.2: Mean CH_4 flux rates (n=4) (a), NH_4^+-N contents (0-30 cm) (b), NO_3^--N contents (0-30 cm) (c), mean CO_2 flux rates (n=4) (d) and gravimetric soil water content (GSW, 0-30 cm) (e) in the slurry injection treatment (INJ) without nitrification inhibitor (NI) and with the NIs DMPP (INJ + DMPP), DMPSA (INJ + DMPSA) and the combination of DMPP and DMPSA (INJ + DMPP & DMPSA) over the two experimental years. For NI abbreviations see Table 5.1. During cropping seasons, soil samples for N_{min} (NO_3^-, NH_4^+) and GSW determination were taken from the slot area. During fallows, soil sampling was conducted randomly from the particular plots. Arrows indicate fertilization measures. Error bars are omitted for the purposes of clarity. Data from NH_4^+-N contents, NO_3^--N contents, CO_2 flux rates and gravimetric soil water content are taken from Chapter 3 and 4.

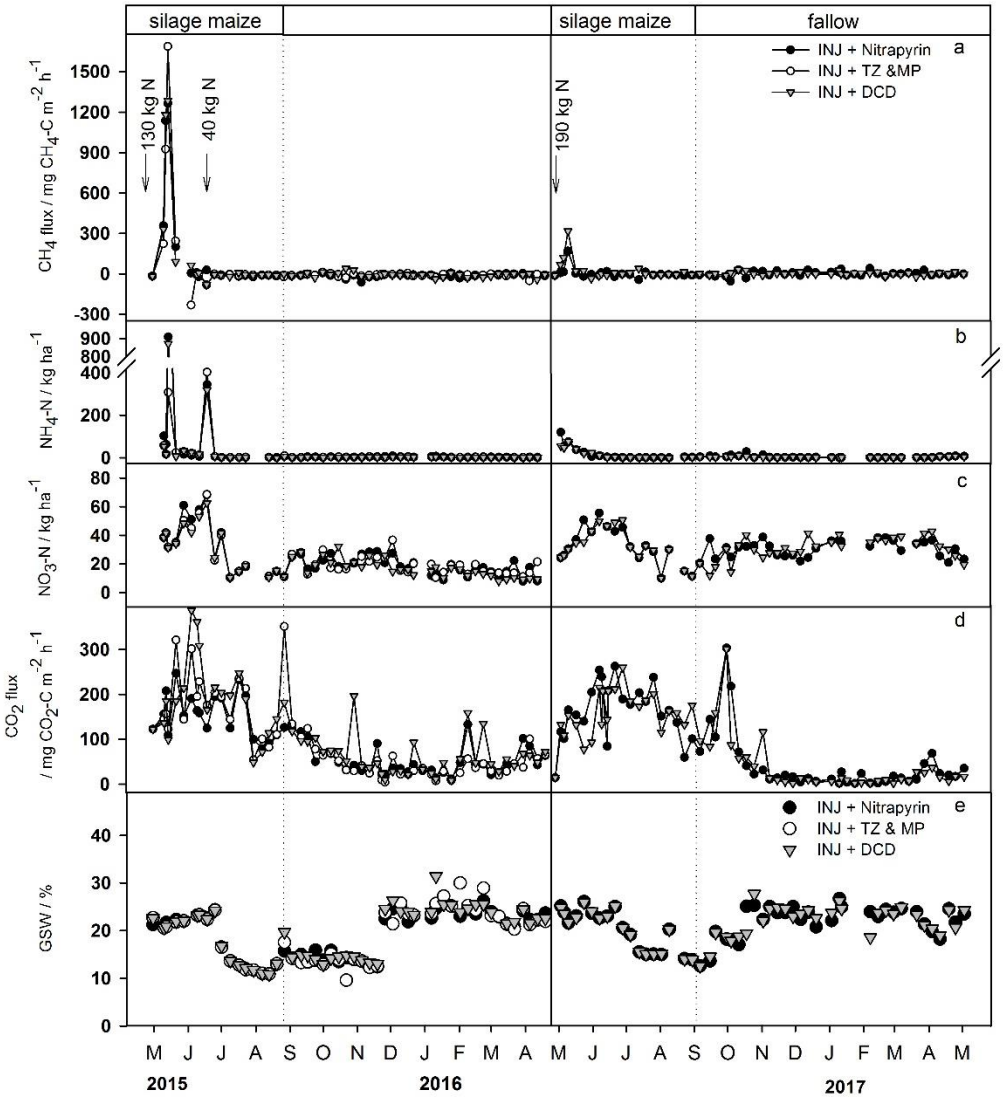

Figure 5.3: Mean CH_4 flux rates (n=4) (a), NH_4^+-N contents (0-30 cm) (b), NO_3^--N contents (0-30 cm) (c), mean CO_2 flux rates (n=4) (d) and gravimetric soil water content (GSW, 0-30 cm) (e) in the slurry injection treatments with the nitrification inhibitors (NIs) Nitrapyrin (INJ + Nitrapyrin), TZ and MP (INJ + TZ & MP) and DCD (INJ + DCD) over the two experimental years. For NI abbreviations see Table 5.1. During cropping seasons, soil samples for N_{min} (NO_3^-, NH_4^+) and GSW determination were taken from the slot area. During fallows, soil sampling was conducted randomly from the particular plots. Arrows indicate fertilization measures. Error bars are omitted for the purposes of clarity. Data from NH_4^+-N contents, NO_3^--N contents, CO_2 flux rates and gravimetric soil water content are taken from Chapter 3 and 4.

5.4.3 Net CH_4 fluxes

In both years, the CON, MIN, INC and INC + DMPP & DMPSA treatments were net CH_4 sink (Figure 5.4). In contrast, all treatments with injected slurry could not offset the high flux rates after fertilization within one entire year resulting in net CH_4 emissions. However, addition of NIs tended to reduce the annual net CH_4 fluxes from slurry injection and incorporation.

Net CH_4 uptake in the first year ranged between 606 g CH_4-C ha^{-1} yr^{-1} (CON) and 1116 g CH_4-C ha^{-1} yr^{-1} (MIN). In the same year, net CH_4 emission varied between 2468 g CH_4-C ha^{-1} yr^{-1} (INJ + DCD) and 5645 g CH_4-C ha^{-1} yr^{-1} (INJ). Despite no statistically significant differences between treatments within the categories net sinks or sources, CH_4 emissions after injection tended to be reduced by NIs. The reduction ranged between 45 % and 56 %.

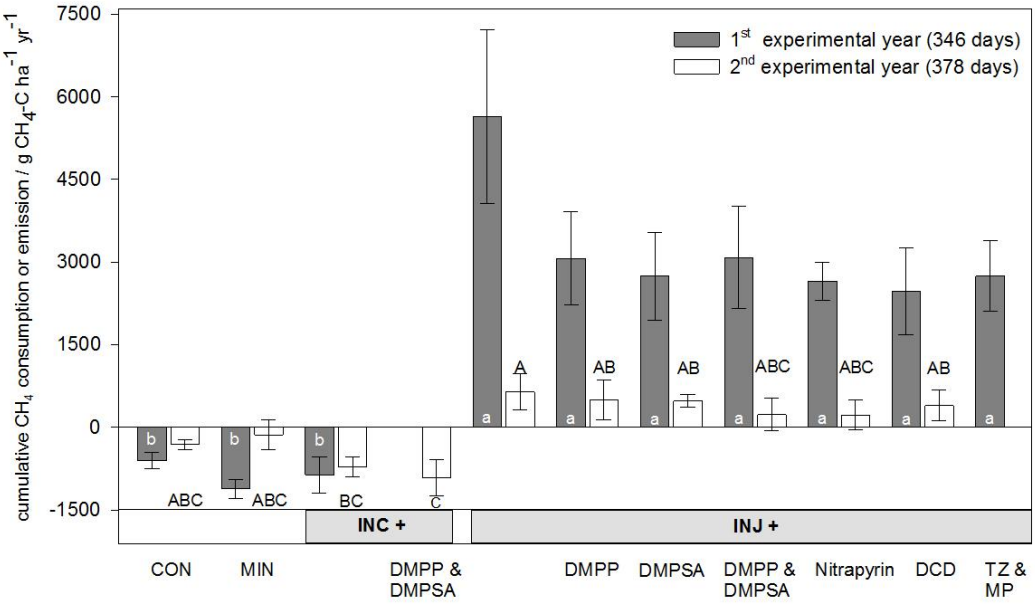

Figure 5.4: Mean cumulative CH_4 balances (*n=4* ± standard errors) as affected by N-fertilizer (no, mineral or organic), application technique (broadcast or injection) and nitrification inhibitor (NI) addition in the first (lower-case letter) and in the second (upper-case letter) experimental year. For treatment abbreviations see Table 5.1. Different letters indicate statistically significant differences between groups within the same year (*Student-Newman-Keuls test*, $p<0.05$).

In the second year, CH_4 consumption as well as production were on a substantially lower level compared to the first investigation year. Although no statistically significant differences among the soils as net CH_4 sinks in the different treatments (CON, MIN, INC, INC + DMPP & DMPSA) could be detected, slurry incorporation tended to induce the highest CH_4 uptakes. Addition of DMPP & DMPSA tended to increase net CH_4 oxidation by a further 20 % in comparison to sole slurry incorporation. This corresponds to the highest CH_4 uptake of the second year (−908 g CH_4-C ha^{-1} yr^{-1}). In contrast, slurry injection again recorded the highest net CH_4 emissions with 643 g CH_4-C ha^{-1} yr^{-1}. However, in the second year, differences between the two categories net CH_4 sinks and sources could not always be statistically secured. For the INJ treatment, exclusively a significant difference from INC treatments (INC, INC + DMPP & DMPSA) could be detected. INJ treatments with NIs were not significantly different from INC treatment due to reduced CH_4 emissions between 23 % to 65 %. In the second year, Nitrapyrin (65

%) as well as the combination of DMPP & DMPSA (64 %) tended to reduce CH_4 emission the most and it was not significantly different from slurry incorporation plus NI (INC + DMPP & DMPSA).

5.5 Discussion

5.5.1 CH_4 fluxes over time

The high spatial and temporal variability as observed here was confirmed repeatedly for a wide range of environments in previous studies and it is mainly caused by varying water table positions, soil temperature and inhomogeneity of microbial activity (*Rothfuss and Conrad,* 1993; *Topp and Pattey*, 1997).

The CH_4 uptake from the unfertilized control characterized the study site as an arable soil with typical activity of CH_4 oxidation (*Hellebrand and Scholz*, 2000; *Topp and Pattey*, 1997).

In contrast to the expected NH_4^+-induced decrease of CH_4 oxidation after broadcast organic or mineral fertilization in other experiments (*Hansen et al.*, 1993; *Hütsch et al.*, 1993), these two treatments did not respond here. The impact of the regulatory factor N-fertilizer on the mechanisms responsible for CH_4 oxidation are still rarely understood (*Mohanty et al.*, 2006). NH_4^+ and NO_3^- fertilization is disputed from no to inhibiting effect on CH_4 oxidation (*Tlustos et al.*, 1998) up to stimulated CH_4 consumption rates (*Bodelier and Laanbroek*, 2004; *Mohanty et al.*, 2006). Thus, in the present study, reasons for the inconsistent responses of CH_4 oxidation after mineral (MIN) or organic (INC) fertilization remain unclear.

In contrast, all treatments with slurry injection responded clearly on fertilization measure with high positive CH_4 flux rates in the periods after injection. Due to the long duration of positive CH_4 fluxes, CH_4 emissions from INJ treatments can be attributed to methanogenesis rather than to volatilization of dissolved slurry CH_4 produced during storage (*Chadwick et al.*, 2000; *Flessa and Beese*, 2000; *Wulf et al.*, 2002b).The conditions promoting CH_4 formation were probably induced by anoxic redox potentials of −100 mV or less (*Flessa and Beese*, 2000; *Masscheleyn et al.*, 1993) due to the high soil moisture statuses in the slot zone and the increased microbial respiration in and next to the injected hot spot (*Comfort et al.*, 1990; *Dosch and Gutser*, 1996; *Flessa and Beese*, 2000). The assumption of anoxic redox potentials after slurry injection was especially reinforced by the strong correlation between decreasing soil NO_3^- contents and positive CH_4 fluxes after slurry injection in 2015. Hence, it may be expected that the reducing soil conditions primary induced NO_3^- reduction with a simultaneous increase of stimulation methanogenesis (*Chidthaisong and Conrad*, 2000; *Le Mer and Roger*, 2001). The

high N_2O flux rates measured in the same period also hint at intense denitrification as a result of O_2 depletion (Chapter 3 and 4).

Moreover, in the period after slurry application, the supply of small organic substrates, essential for methanogens, might be guaranteed through microorganisms metabolizing substrates, such as slurry derived organic carbon (*Le Mer and Roger*, 2001; *Yagi and Minami*, 1990).

In the course of time, after fertilization-induced changes in CH_4 dynamics, the soil was predominantly a CH_4 sink for all treatments. Although not statistically significant, CH_4 oxidation rates tended to be influenced stronger by soil moisture status than by soil temperature as observed by other authors (*Topp and Pattey*, 1997; *van den Pol-van Dasselaar et al.*, 1998).

The use of NIs neither affected CH_4 dynamics from slurry incorporation nor from slurry injection significantly. However, in comparison to sole slurry amendments, slurry fertilization with NIs tended to induce lower CH_4 emission (after injection) or higher CH_4 uptake (after broadcast incorporation). This observation might indicate a higher O_2 availability through NIs probably caused by lower nitrification rates (*Van Nguyen et al.*, 2017). The differing NIs investigated showed no distinct trends concerning the reduction of CH_4 fluxes. Thus, the NI-induced effect may rather be attributed to a generally increase of redox potential (*Van Nguyen et al.*, 2017) than to any inhibitor-specific characteristic.

Comparing the CH_4 dynamics from both years, it can be noted that CH_4 uptake and CH_4 emission were generally higher in the warmer and dryer first year than in the second one. These differences were also reflected in the height of cumulative CH_4 fluxes from net CH_4 sinks (CON, MIN, INC, INC + DMPP & DMPSA) and net CH_4 sources (all INJ treatments) (Figure 5.4). Despite no statistical difference among the treatments with net CH_4 uptake, reduced bulk density in the INC treatments, induced by additional harrowing for slurry incorporation, may have increase CH_4 and O_2 diffusion into the soil (*Hansen et al.*, 1993; *Ruser et al.*, 1998; *Striegl*, 1993). This effect was especially evident in the wetter second year, in which soil moisture seemed to be the limiting factor for soil aeration and thus for CH_4 oxidation (*Dunfield et al.*, 1995; *Lessard et al.*, 1994).

In contrast to slurry harrowing which even tended to enhance CH_4 uptake, slurry injection promoted CH_4 formation strongly as has been reported already in previous studies (*Flessa and Beese*, 2000; *van den Pol-van Dasselaar et al.*, 1999; *Wulf et al.*, 2002). Comparing the two experimental years, the longer duration and the generally higher level of positive CH_4 fluxes after slurry injection in 2015 suggest that methanogenesis was promoted stronger in the first than in the second year. On the one hand, it is conceivable that the higher soil temperatures after slurry injection in the first year promoted

methanogenesis stronger than in the cooler second year (*Dunfield et al.*, 1993). On the other hand, it may be expected that under wetter conditions, methanogenesis was limited indirectly by limited substrate availability for CH_4 formation. For methanogenesis, the supply of substrates such as small organic substrates or CO_2 is essential for CH_4 formation. However, when soil respiration is limited under wet soil conditions through O_2 depletion, the substrate provision through microorganisms is reduced likewise due to low degradation rates. Thus, methanogenesis might be limited by reduced substrate availability caused by low degradation rates of organisms which have previously blocked the complex reaction chain of CH_4 formation (*Topp and Pattey*, 1997).

A negative correlation between soil moisture status and microbial respiration from INJ treatments in the period after fertilization in 2016 (r^2=0.36, Pearson Product Moment Correlation, $p<0.05$) and a positive correlation between these two variables after injection in 2015 (r^2=0.14, Pearson Product Moment Correlation, $p<0.05$) reinforced this assumption.

Consequently, putative O_2 depletion in the second year induced by high soil moisture, was probably the key factor for limiting CH_4 production as well as for CH_4 oxidation.

5.6 Conclusion

Concerning atmospheric burden, the use of NIs tended to have a beneficial effect in both years due to reducing CH_4 emission from slurry injection (in mean by 52 % and 43 % in the first and second year, respectively) or even tended to increase CH_4 consumption by 20 % after slurry incorporation which was investigated in the second year. Thus, beside the potential of reducing fertilization-induced N_2O emissions at this study site as reported in Chapter 4, the present results have shown that NIs might also help to improve the CH_4 budget in silage maize production. Moreover, enhanced aeration induced by additional harrowing from slurry incorporation also tended to increase CH_4 oxidation, especially under conditions with higher soil moisture status. In contrast, placed slurry application by injection without NI created the most suitable conditions for CH_4 formation.

Hence, a reduced greenhouse gas release from cropping systems at this study site is conceivable through the creation of soil conditions more favorable for CH_4 consumption or unsuitable for CH_4 formation by soil loosening and/or using NIs.

In contrast to the expected NH_4^+-induced decrease of CH_4 oxidation after broadcast organic or mineral fertilization in other experiments, these two treatments did not response here.

Nevertheless, further microbiological research is needed to enable a better understanding of how these or other management factors (*e.g.* N-fertilizer form, fertilizer application technique, NI addition) regulate CH_4 dynamics.

5.7 Acknowledgments

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6 General Discussion

Against a background of constant world population growth, a further substantial nitrogen (N) increase in cropping systems through chemical and organic N-fertilizers or biological N fixation will be necessary (*Smil*, 1999). However, to prevent a simultaneous increase of reactive N compounds which contribute to climate change, acidification, eutrophication and involve extra cost for N-fertilizers, fertilization strategies reducing N losses and enhancing nitrogen use efficiency (NUE) are urgently required (*Dawson et al.*, 2008; *Smil*, 1999). Thus, agricultural systems aim for a crop production with minimum total greenhouse gas (GHG) release and optimized internal nutrient cycles (*Flessa et al.*, 2002; *UBA*, 2017).

For the final evaluation of GHG mitigation strategies from management in silage maize production on a medium textured soil in Southwest Germany, the effects of fertilizer form, application technique and nitrification inhibitors (NIs) on area- and yield-related GHG balances are summarized in Table 6.1. In the following, it is discussed how single management strategies might control the respective group contributing to GHG balance.

6.1 Gaseous N losses (N_2O, NH_3)

Over all treatments, nitrous oxide (N_2O) emissions had the highest share on each GHG balance in both years. Generally, the background emissions emitted from unfertilized control plots (CON) characterized our experimental field as a site with conditions favorable for N_2O formation due to annual emissions two- or threefold as high as the general assumption of 1 kg N_2O-N ha^{-1} $year^{-1}$ (*Bouwman*, 1996). Despite these high background emissions, carbon (C) seemed to be limited for N_2O formation. This limiting effect was probably caused by the depletion of reductants in the top soil layer (*Giles et al.*, 2012; *Weier et al.*, 1993). A strong C-limitation for denitrifying microorganisms at this study site was already confirmed by *Pfab* (2011) in a vegetable crop rotation. Since N crop demand was calculated on the basis of total N, the amount of inorganic N applied was twice as much in the mineral as in the organically fertilized treatments, thus potentially reinforcing the C limitation in the mineral treatment.

Consequently, the N losses from the mineral and organically fertilized treatments in this study were hardly comparable. Nevertheless, results from *Snyder et al.* (2009) also indicated, that mineral N-fertilizers lower GHG balance and N_2O emissions when compared to organic N-fertilizers. However, when C was no limiting factor for biological N_2O production, several studies confirmed similar N_2O release independent of N source (*Pelster et al.*, 2012; *Velthof and Mosquera*, 2011). Nevertheless, mineral fertilization with calcium ammonium nitrate (CAN) has also a substantially lower risk of ammonia (NH_3) losses than organic fertilization (*Clemens et al.*, 2006; *Rösemann et al.*, 2017),

Table 6.1: Mean area- and yield-related greenhouse gas (GHG) emissions and GHG sources as affected by application technique, nitrification inhibitor (NI) addition and year. Values in brackets indicate the percentage of the single sources to total GHG emission. Different superscript letters indicate statistically significant differences between treatments within the same year and column (*Student Newman Keuls* for dataset from first (*ANOVA on Ranks*; *p=0.007, n=4*) and second year (*ANOVA*; *p<0.05, n=4*)).

Yr	Treatment	GHG sources										GHG emissions			
		N_2O emissions		NH_3 emissions		NO_3^- leaching		CH_4 fluxes		CAN fertilizer		Fuel consumption		Area -related	Yield -related
		[kg CO_2 eq. ha^{-1} yr^{-1}] (percentage of single source to total GHG emission)												[kg CO_2 eq. ha^{-1} yr^{-1}]	[kg CO_2 eq. Mg^{-1} DM^{-1}]
1st	CON	1067.1	(83.4)	0.0	(0.0)	32.2	(2.5)	-18.6	(-1.5)	0.0	(0.0)	198.9	(15.5)	1279.6^{c}	105.3^{c}
	MIN	1479.6	(59.3)	0.1	(<0.1)	222.8	(8.9)	-34.2	(-1.4)	624.6	(25.0)	202.0	(8.1)	2494.8^{b}	166.3^{b}
	INC	982.1	(65.2)	65.1	(4.3)	213.3	(14.2)	-26.4	(-1.8)	0.0	(0.0)	272.0	(18.1)	1505.9^{c}	121.7^{c}
	INJ	7598.8	(91.3)	45.5	(0.5)	217.7	(2.6)	173.1	(2.1)	0.0	(0.0)	288.9	(3.5)	8324.0^{a}	582.4^{a}
	INJ+DMPP	5988.3	(90.3)	45.5	(0.7)	214.0	(3.2)	94.1	(1.4)	0.0	(0.0)	288.9	(4.4)	6630.8^{a}	570.6^{a}
	INJ+DMPSA	5805.5	(90.1)	45.5	(0.7)	219.0	(3.4)	84.2	(1.3)	0.0	(0.0)	288.9	(4.5)	6443.2^{a}	456.2^{a}
	INJ+DMPP&DMPSA	4508.4	(87.4)	45.5	(0.9)	219.0	(4.2)	94.5	(1.8)	0.0	(0.0)	288.9	(5.6)	5156.3^{a}	345.0^{a}
	INJ+Nitrapyrin	6002.2	(90.4)	45.5	(0.7)	223.3	(3.4)	81.3	(1.2)	0.0	(0.0)	288.9	(4.4)	6641.1^{a}	433.2^{a}
	INJ+DCD	5146.0	(89.1)	45.5	(0.8)	219.7	(3.8)	75.7	(1.3)	0.0	(0.0)	288.9	(5.0)	5775.8^{a}	391.5^{a}
	INJ+TZ&MP	6277.8	(90.8)	45.5	(0.7)	220.1	(3.2)	84.1	(1.2)	0.0	(0.0)	288.9	(4.2)	6916.5^{a}	511.7^{a}
2nd	CON	1543.4	(88.2)	0.0	(0.0)	25.9	(1.5)	-9.6	(-0.5)	0.0	(0.0)	191.2	(10.9)	1750.9^{C}	163.4^{B}
	MIN	2881.6	(71.7)	0.1	(<0.1)	249.1	(6.2)	-4.2	(-0.1)	698.1	(17.4)	194.4	(4.8)	4019.1^{B}	222.5^{B}
	INC	3128.5	(85.6)	58.6	(1.6)	236.7	(6.5)	-22.2	(-0.6)	0.0	(0.0)	254.9	(7.0)	3656.6^{BC}	250.0^{B}
	INC+DMPP&DMPSA	2536.0	(83.0)	58.6	(1.9)	232.5	(7.6)	-27.8	(-0.9)	0.0	(0.0)	254.9	(8.3)	3054.1^{BC}	240.9^{B}
	INJ	5365.8	(90.0)	58.8	(1.0)	240.2	(4.0)	19.7	(0.3)	0.0	(0.0)	278.7	(4.7)	5963.3^{A}	392.6^{A}
	INJ+DMPP	2572.4	(81.3)	58.8	(1.9)	238.8	(7.5)	15.2	(0.5)	0.0	(0.0)	278.7	(8.8)	3163.9^{BC}	208.2^{B}
	INJ+DMPSA	3923.5	(86.8)	58.8	(1.3)	242.0	(5.4)	14.7	(0.3)	0.0	(0.0)	278.7	(6.2)	4517.6^{B}	284.5^{AB}
	INJ+DMPP&DMPSA	2293.9	(79.7)	58.8	(2.0)	238.4	(8.3)	7.0	(0.2)	0.0	(0.0)	278.7	(9.7)	2876.9^{BC}	183.6^{B}
	INJ+Nitrapyrin	3677.7	(86.3)	58.8	(1.4)	239.1	(5.6)	6.9	(0.2)	0.0	(0.0)	278.7	(6.5)	4261.2^{B}	280.4^{AB}
	INJ+DCD	2356.4	(80.0)	58.8	(2.0)	238.6	(8.1)	12.2	(0.4)	0.0	(0.0)	278.7	(9.5)	2944.7^{BC}	199.4^{B}

CON= unfertilized control; MIN= mineral fertilization with calcium ammonium nitrate; INC= cattle slurry incorporation; INJ= cattle slurry injection; for NI abbreviations see Table 4.1; CO_2 eq.= CO_2 equivalents. CO_2 eq. were calculated according to the *IPCC* (2007) guidelines. Before conversion into CO_2 equivalents, annual N_2O emissions from fertilized treatments were not corrected for background emission.

however an exclusively mineral fertilization is not an option for systems with livestock farming.

Thus, strategies to reduce gaseous N losses after application of organic fertilizers are of particular importance and still required. How application technique affects gaseous N losses was intensively investigated and reviewed over the last decades. Thereby, application method was identified as one of the main influencing factors on field-related gaseous N release. In review articles from *Chadwick et al.* (2011), *Langevin et al.* (2010), *VanderZaag et al.* (2011) and *Webb et al.* (2010), the authors concluded that slurry injection for at least 10 cm depth is the most efficient way of lowering NH_3 emissions but it might also promote N_2O formation stronger than any other application technique (*e.g.* splash plate, trail hose, harrowing, shallow injection). Similar to these observations, the results of the present study have shown that the beneficial effect of slurry injection in comparison to broadcast incorporation concerning NH_3 release might be offset by an exorbitantly exceedance of N lost as N_2O. In particular, the N_2O emissions from slurry injection at our study site was in the upper range of N_2O emissions reported from other study sites (*Langevin et al.*, 2010; *Velthof and Mosquera*, 2011) and even exceeded the upper uncertainty range from the *IPCC* (2006) emission factor (EF) of 3 %.

Thus, in accordance with the reviews mentioned above, the results of the present study have evaluated banded slurry application by trailing hose with immediate incorporation as the most suitable application technique concerning reduction of gaseous N losses (N_2O and NH_3 emissions) in total, in comparison to any other application technique. However, its compromise between N_2O and NH_3 reduction indicates further options optimizing broadcast slurry incorporation.

However, as listed in Chapter 2, most of the mitigation strategies for N_2O (Table 2.2) and NH_3 emissions (Table 2.3) were conflicting: *e.g.* manure dilution was recommended for reducing NH_3 volatilizations but might provoke N_2O formation strongly due to decreasing dry matter (DM) content (*VanderZaag et al.*, 2011). Similarly, acidification was promoted to decrease NH_3 losses but bears the risk of increasing N_2O production through a shift of the N_2/N_2O ratio towards higher N_2O portions with decreasing pH (*VanderZaag et al.*, 2011).

In order not to compromise between optimal reduction of NH_3 or N_2O, the present study aimed to investigate the combination of less weather-dependent NH_3 mitigation through slurry placement into the soil by injector and N_2O reduction through the use of NIs by intervening in N_2O formation via denitrification through desynchronizing C and N availability in the injection slot.

This idea was not new (*Comfort et al.*, 1990), however, previous studies usually observed solely up to two NIs and frequently covered only short sampling periods of a few

weeks where absolute N_2O emissions and the percentage of NI-induced N_2O mitigation remained uncertain. Moreover, the observation of more than two NIs allowed a comparison of numerous marketable or not yet marketable NIs under the given conditions.

Generally, a N_2O reduction from field-related emissions by one-third through the use of NIs is assumed (*Ruser and Schulz*, 2015; *Smeets et al.*, 2009). In the present study, this expectation could be confirmed by reducing N_2O emissions from slurry injection by 36 % (mean over all treatments and years). However, the N_2O reduction potentials from injection tended to differ between NIs and years which was reflected by a broad range (17 % to 61 %). It is assumed that this variation was mainly as a result of different release behavior and/or persistence of active compounds. During the investigations, DMPP and DCD tended to be characterized as inhibitors with the highest inhibitory effect in the early period after application. In contrast, DMPSA, Nitrapyrin and TZ & MP tended to have their highest inhibitory effect at a later period, probably due to delayed release behavior. Consequently, the combination of DMPP & DMPSA covered the longest inhibition timeframe due to combining the earlier and delayed inhibitory maximum.

However, the inhibitory duration and effectiveness of the particular NI was related to environmental conditions, such as soil temperature or soil moisture. As observed in the first year, under warmer soil conditions favoring NI degradation (*Chen et al.*, 2010; *Irigoyen et al.*, 2003; *Slangen and Kerkhoff*, 1984; *Subbarao et al.*, 2006; *Zerulla et al.*, 2001), NIs with potential delayed release behavior had an advantage concerning N_2O mitigation over inhibitors with an already decreasing inhibitory effect later in the course of time.

In the second year, cooler soil conditions extended the timeframe of efficient inhibition from NIs with putative early inhibitory maximum and postponed the maximum inhibitory effect of NIs with delayed release behavior, probably due to a lower degradation rate.

A heavy rainfall event favoring denitrification occurred four weeks after fertilization in the first and also in the second year. After this rewetting, in contrast to the warmer first year, injection treatments containing NIs with an earlier inhibition maximum (DMPP, DMPP & DMPSA, DCD) resulted in a more efficient N_2O reduction than NIs with a delayed inhibitory effect (DMPSA, Nitrapyrin).

Although all NIs decreased the mean fertilization-induced N_2O release from slurry injection over both years by an order of magnitude comparable to slurry incorporation, the combination of DMPP & DMPSA tended to have the most beneficial effect on reducing N_2O release in both years. This effect was probably caused by the combination of the

earlier (DMPP) and delayed (DMPSA) release behavior of the active compound dimethylpyrazole (DMP), resulting in prolonged ammonium (NH_4^+) stabilization.

Against the background of increasing extreme weather events, such as distinct drought periods or heavy rainfalls as result of climate change (*UBA*, 2011), mitigation strategies with low weather-dependence are of particular importance. Especially in Southwest Germany, which today is already a comparatively warm region in Germany, NIs with high persistence under warm soil conditions might be important in the future.

Thus, based on the present observations, the combination of DMPP & DMPSA turned out as the most appropriate NI for slurry injection due to its low potential to be influenced by unpredictable environmental factors. Moreover, in an additional treatment, the effect of this NI combination plus slurry incorporation was investigated in the second year only. Here, there was a tendency for NIs to reduce the annual N_2O release by 20 % in comparison to slurry incorporation alone, demonstrating the potential of further optimization broadcast slurry incorporation.

6.2 Methane (CH_4)

Despite the comparable low and non-significant impact of CH_4 fluxes on total GHG release, differences between cumulative CH_4 fluxes of the varying fertilization strategies were obvious and indicated the option of improving CH_4 budget in silage maize production. In both years, plots from unfertilized control (CON), mineral fertilization (MIN) and broadcast slurry incorporation (INC) treatments were net CH_4 sink.

CH_4 uptake from unfertilized control characterized the study site as an arable soil with typically low activity of CH_4 oxidation (*Hellebrand and Scholz*, 2000; *Topp and Pattey*, 1997). In contrast to the expected NH_4^+-induced decrease of CH_4 oxidation after broadcast organic or mineral fertilization (*Clemens et al.*, 2006; *Hansen et al.*, 1993; *Hütsch et al.*, 1993), NH_4^+ application by mineral N or by slurry broadcast incorporated did not decrease net CH_4 uptakes.

Although not statistically significant, enhanced aeration induced by additional harrowing from slurry incorporation tended to increase CH_4 oxidation, especially under conditions with higher soil moisture status.

In contrast, all treatments with slurry injection responded clearly on fertilization measure with high positive CH_4 flux rates in the periods after injection. The long duration of positive CH_4 fluxes after injection were an indicator that CH_4 emissions were rather as a result of methanogenesis than volatilization of dissolved slurry CH_4 produced during storage (*Chadwick et al.*, 2000; *Flessa and Beese*, 2000; *Wulf et al.*, 2002). This could also be confirmed in the present study by *Gassner* (2017) (refer to Figure 10.3). The

high flux rates induced through injection could not be offset within one entire year and resulted in net annual CH_4 emissions.

However, addition of NIs tended to reduce the annual net CH_4 fluxes from slurry injection by approximately one-half (mean over all treatments and years) or even tended to increase CH_4 consumption by 20 % after slurry incorporation (investigated in the second year). This observation might be attributed to higher oxygen (O_2) availability in periods after fertilization due to inhibited nitrification (*Van Nguyen et al.*, 2017).

However, no obvious pattern for the height of CH_4 reduction or a relationship between N_2O and CH_4 reduction from differing NIs was distinguishable. Thus, the NI-induced effects may rather be attributed to a generally increase of the redox potential (*Van Nguyen et al.*, 2017) than to any inhibitor-specific characteristic.

Comparing CH_4 dynamics from both years, CH_4 uptake as well as CH_4 emission was generally higher in the warmer and dryer first year than in the second one. Putative O_2 depletion in the second year induced by high soil moisture, was probably the key factor for limiting CH_4 production and CH_4 oxidation.

6.3 Nitrate (NO_3^-)

NO_3^- losses through leaching or runoff also contribute to indirect N_2O emissions (*VanderZaag et al.*, 2011). Thus, NO_3^--induced N_2O emissions were also included in this GHG balance.

These differed only slightly because they were calculated according to the *IPCC* (2006) guidelines using N-fertilization amount (which was the same for all fertilized treatments) and N content in crop residues. However, fertilizer form, application technique or the use of NIs were not taken into account.

In comparison to mineral fertilizer form, organic fertilizers are similar or less susceptible for NO_3^- leaching (*Daudén and Quílez*, 2004). The authors attributed the lower risk of NO_3^- leaching losses from organic fertilizers to immobilization of slurry derived ammonium (NH_4^+) and to fixation of slurry NH_4^+ in the interlayers of clay minerals. In contrast, *Thomsen et al.* (1997) attributed a higher risk of leaching losses to organic fertilizers due to mineralization of organically bounded N in periods with low N demand.

Up to now, the effect of slurry application technique on NO_3^- leaching losses has rarely been investigated. *Weslien et al.* (2006) observed no significant differences between slurry harrowing and injection. But a lower risk of NO_3^- leaching from broadcast harrowing in comparison to injection is conceivable due to higher soil-slurry interactions (*Sørensen and Amato*, 2002).

Despite repeated confirmation that NIs may reduce NO_3^- leaching losses through delaying nitrification and thus ensure optimal N supply with rising crop demand (*Di and Cameron*, 2012; *Ruser and Schulz*, 2015), effects of NIs could not be taken into account for calculations of NO_3^- leaching in the present study. However, at this study site, increased NO_3^- leaching losses indirectly induced through the use of NIs are similarly conceivable.

Even though the study site was characterized as soil with reduced risk of NO_3^- leaching during maize cropping season due to its medium heavy soil texture (*Gaines and Gaines*, 1994; *Sutton et al.*, 1986), the reduction of gaseous N losses through NI addition might result in increased NO_3^- content in soil and leachate. This expectation requires that N not used by plant uptake or not lost as NH_3 or N_2O would increase the potential of NO_3^- leaching losses.

Results from a sunflower experiment (refer to Table 10.1) reinforced this assumption of increased N supply when NIs were added to slurry fertilization one year before sunflower cultivation. But how the real share of NO_3^- losses on GHG balance is affected by NIs as well as by application technique or fertilizer form at this study site remains unclear due to the lack of available data.

6.4 Fertilizer production

For calculation of field-related GHG release, emissions from the supply of animal manures were excluded because these pre-chain emissions were accounted for in the sector animal production (animals, housing and storage (*Rösemann et al.*, 2017)). In contrast, pre-chain emissions from imports of production goods, such as mineral fertilizer production and transport, have to be included in GHG balance on field-scale (*Olesen et al.*, 2006; *UBA*, 2017). Consequently, the supply of organic fertilizers has an alleged advantage in comparison to suppling mineral fertilizers, at least on field-scale.

6.5 Fuel consumption and man power

Fuel consumption from mineral fertilizer application was marginal in comparison to organic fertilizer application. This was mainly caused by fertilizer placements into soil. Moreover, due to the two tractor passages of broadcast slurry incorporation (surface application and subsequent incorporation), fuel consumption of this application was additionally increased as well as the need for manpower.

Despite the fact that slurry injection needs less manpower due to less tractor passages, this application had a generally higher tractive power requirement on the silty loamy soil resulting in slightly higher GHG emissions attributed to fuel consumption than surface slurry placement with subsequent incorporation (*KTBL*, 2009).

6.6 N removal and yield response

Although N removal was not included directly for calculations of GHG balances, an indirect impact concerning atmospheric burden was given by the N amount bounded in maize plants affecting N_{min} availability in soil during cropping season (reduced N available for N losses with rising N uptake of growing maize plants) and after harvest (N removed from field, mineralization of remaining crop residues).

Moreover, in contrast to area-related GHG emissions, yield-related GHG releases are decisive for an expressive evaluation of process optimization from silage maize production. Even though the total N applied was equal for all fertilized treatments in the respective study years, the amounts of immediately plant-available N applied was twice as high in mineral fertilized treatments as in organically fertilized treatments. These discrepancies resulted in significantly higher N removals from mineral fertilization in comparison to organically fertilized treatments. However, N removal and DM yield was positively affected by slurry placement near the maize roots in comparison to broadcast incorporation. Contrary to our expectations, neither maize yield nor N removal was affected by any of the NIs investigated. This was probably a result of N doses exceeding N demand of maize plants and thus masking the beneficial effect of NIs. The low NO_3^- leaching potential during the cropping season of this study site might also have contributed to this effect.

Yield-related and area-related GHG balances of the different treatments were in a relatively similar order. Exclusively the MIN treatment in the second year reached a significant improvement in GHG balance through yield-relation.

However, as the mineral-fertilizer N equivalents (MFE) already indicated, a substantial share of slurry derived N was still organically bounded (refer to Chapter 3.4.5). An additional field experiment on the study site from the first year reinforced the residual effect from slurry N and NI addition. Therefore, sunflower was cultivated without any fertilization measure one year after treatment-specific fertilization. Since prior fertilization was conducted with NIs, improved N removals in sunflower head and crop at flowering and physiological maturity indicated a higher N supply. This putative enhanced N availability could be statistically secured for N removal of sunflower head and crop at flowering when prior slurry injection was conducted with DCD (Table 10.1). At physiological maturity, slurry injection with TZ & MP and DMPP increased N removals in sunflower heads whereas the DMPP also recorded significant higher N removal in crop compared to sole slurry injection one year earlier.

Alonso-Ayuso et al. (2016) established a similar field experiment with prior mineral fertilization with and without NI. They attributed the higher sunflower N removal from treatments with NI addition in comparison to single fertilization to N conservation in

non-ready soil available forms during at least one year and subsequent release to meet sunflower crop demand.

In contrast to expectations on the basis of previous studies (*Webb et al.*, 2010), results from organically fertilized treatments neither generated any obvious pattern between N losses and N removal from silage maize nor between N losses and N supply in sunflowers.

6.7 Conclusion

Despite the higher amount of inorganic N applied, mineral fertilization (MIN) proved to be an appropriate fertilization method for farms without animal husbandry and soils limiting denitrification rates by C depletion (Figure 6.1).

Sole slurry injection (INJ) turned out to be the most suboptimal fertilization strategy for livestock farmers in this experimental region with loamy soils. Despite this application technique having several advantages (*e.g.* lower risk of NH_3 losses, less manpower, improved N removals and yields), the beneficial effects were offset by exorbitantly high atmospheric burden, mainly caused by direct N_2O emissions.

Against the risk of increased NH_3 volatilization, trail hose application with immediate harrowing (INC) seemed to be the most recommendable application technique for cattle slurry with no significantly decreased yield or N removal in comparison to injection near the maize roots.

The use of NIs improved GHG balance by reducing N_2O and CH_4 budget from slurry incorporation as well as from slurry injection. This NI-induced GHG mitigation from slurry injection (INJ + NI) resulted in a significantly reduced mean GHG budget over both years by an order of magnitude comparable to slurry incorporation. Therefore, a combination of two NIs, one with relatively easy and one with poor biological degradability, was proven as the most efficient way reducing GHG release.

As shown in the present study, under warmer and dryer soil conditions in the period after fertilization, broadcast slurry incorporation was the preferable application technique despite the increased risk of NH_3 losses. This was mainly caused by non-significant reduction of N_2O releases from slurry injection by the use of NIs, probably due to high degradation rates of the active compounds under warmer soil conditions.

Conversely, under cooler soil conditions less promoting NI degradation and a generally higher soil moisture favoring denitrification, NIs performed their full beneficial effects regarding atmospheric burden. They reduced GHG balances at least to the level from broadcast fertilization or were even comparable with the level of unfertilized control plots.

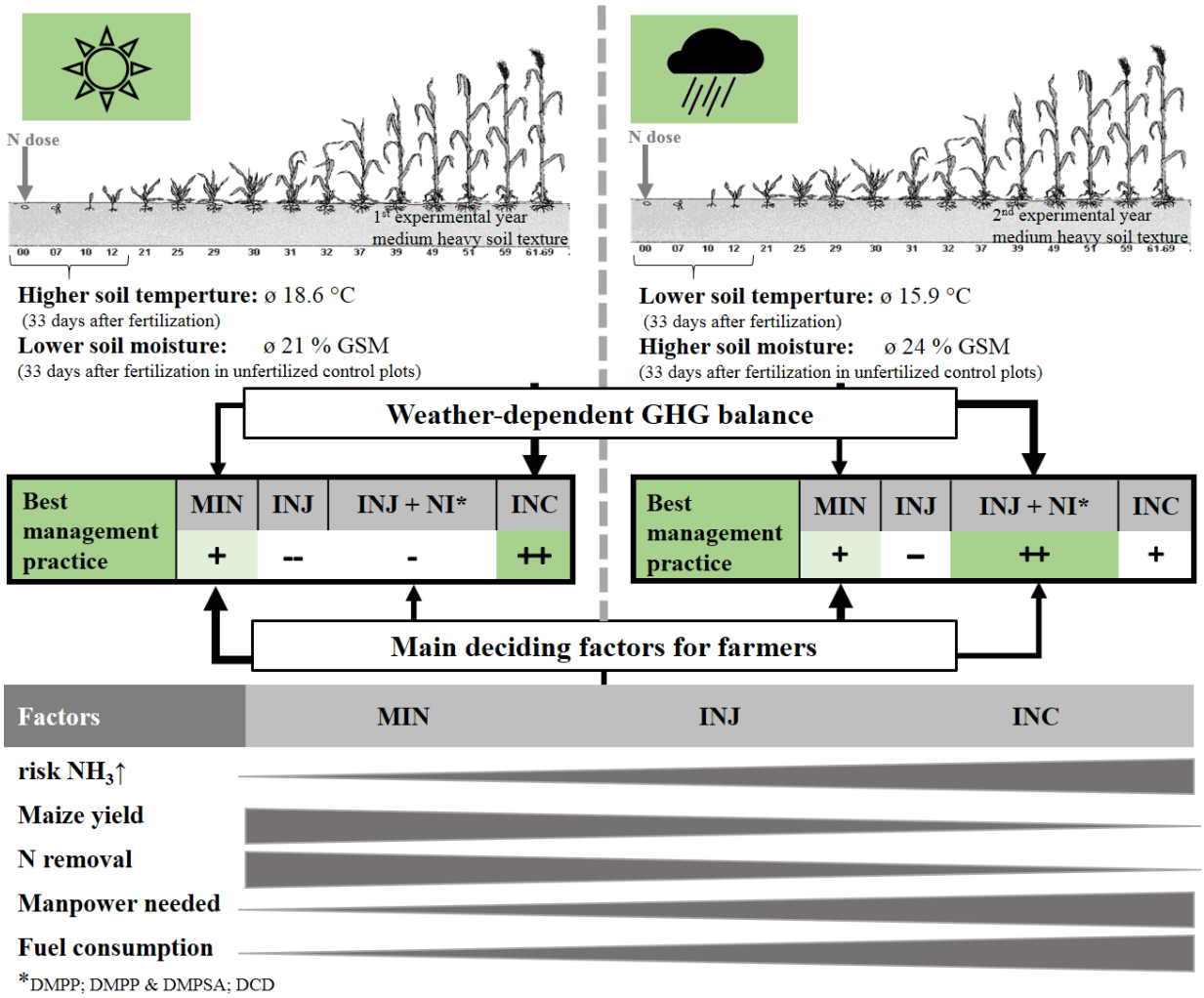

Figure 6.1: Evaluation of fertilization strategies for silage maize production in Southwest Germany on the basis of weather-dependent greenhouse gas (GHG) releases and important deciding factors for farmers. MIN= mineral fertilization; INC= surface cattle slurry application with subsequent incorporation; INJ= cattle slurry injection; NI= nitrification inhibitor; GSM= gravimetric soil moisture content; NH_3= ammonia; N= nitrogen. For further abbreviations see Table 4.1.

Comparing weather conditions of the two experimental years with long-term means for this study site, the wetter and cooler conditions in the period after maize fertilization, showed a much higher correspondence with the long-term average. Consequently, slurry injection with one of the NIs recommended should be preferable.

However with regard to changing climate conditions in Southwest Germany, resulting in a more frequent occurrence of heat waves or heavy rainfalls during maize cropping seasons (*UBA*, 2011), it is difficult to make future forecasts.

Beyond the background of rising air temperatures (*UBA*, 2011) and resulting increased soil temperatures in spring (period of maize seeding), it remains unclear whether NH_3 emissions in the time before broadcast incorporation or a reduced NI efficiency after injection will have a higher impact on total N losses and GHG balance.

But, through an increase of warmer periods during fertilization, it is likely that slurry injection will gain in importance due to lower temperature-dependence of NH_3 emissions. However, this requires a substantially reduced GHG balance from this fertilization method. Consequently, the use of NIs might boost the relevance of injection under warmer climatic conditions in future. Thereby, the degradability of NIs used and the temperature run might be the key factors for the success of this fertilization strategy.

Independent of earlier sowing date and application technique, a general use of NIs should be considered to minimize GHG release when organic fertilizers are inserted into soil.

6.8 Relevance of the GHG mitigation strategies on national base

Against the background of intense animal husbandry and biogas production in Germany, the associated expansion of silage maize cultivation and organic fertilizer supply requires strategies for mitigation GHG release and N losses from maize fields, especially for organic fertilization. Silage maize area in Germany increased from $1.2 \cdot 10^6$ ha in 2003 to $2.1 \cdot 10^6$ ha in 2017 (*DMK*, 2017, 2004). 6.5 % of this area was located in the federal state Baden-Wuerttemberg (*DMK*, 2017). It is challenging to recommend a fertilization strategy as accurate solution on national base.

However, as already confirmed for the use with mineral fertilizers in previous studies (*Ruser and Schulz*, 2015; *Smeets et al.*, 2009), the use of NIs reduced total area-related GHG releases from slurry fertilized treatments by approximately one-third (mean over all NIs, years and application techniques).

In order to evaluate the potential GHG mitigation through NIs on national base, a general reduction of 30 % was assumed in the following; in Germany, the *UBA* (2017) quoted national N_2O emissions in 2015 to $39 \cdot 10^6$ Mg CO_2 equivalents $year^{-1}$ from which 81 % were attributed to agricultural sector.

The assumed NI-induced N_2O reduction might mitigate total national N_2O emissions by 8.2 % for mineral and by 4.5 % for manure fertilization. This is equivalent to a reduction of the total national GHG budget by 0.3 % and 0.2 % through the use of NIs together with mineral and organic manure fertilizers, respectively. To make these percentages more vivid, the putative NI-induced GHG saving of both fertilizer forms related to GHG per capita living in Baden-Wuerttemberg in 2015, is equal to annual GHG emissions of more than half a million people (assuming 7.2 Mg CO_2 equivalents $year^{-1}$ per capita (*LUBW*, 2017)). However, these calculations are associated with high uncertainty due to different GHG mitigation potentials of varying NIs are affected by environmental conditions.

Nevertheless, it is too early to draw final conclusions regarding concrete GHG mitigation potentials through NI addition from previous research, especially due to the high uncertainty about total GHG release from arable soils.

Thus, further research is needed to reduce the current limitation of C accounting tools and emission factors. With an evidence based dataset, the currently missing integration of the complexity of management and environmental factors influencing GHG releases could be implemented (*Chadwick et al.*, 2011).

6.9 Further need of research

Annual GHG measurements (including at least N_2O, CH_4) are a necessary prerequisite reducing the uncertainty of GHG releases from agricultural soils in regions with winter frost. Beside the goal of increasing data accuracy of direct N_2O emissions, especially for organic fertilizers, integrated measurements of NH_3 and N_2O emission in dependence on application technique and the use of NIs is desirable for better understanding of potential tradeoffs between these gaseous N losses.

Moreover, future research should also focus on GHG release induced through residual effects from continuous organically fertilization and/ or the long-term use of NIs. For manure fertilizers, this is of particular importance due to the high share of fertilizer N organically bounded and the requirement of mineralization before N_2O formation is feasible. The use of NIs might reinforce the residual effect from slurry application, as shown in the sunflower experiment (refer to Table 10.1), and thus also requires further research including fields under long-term management conditions.

Generally, the effect of long-term slurry application on N_2O emissions is controversial. Some studies reported higher N_2O reductase activity as a result of increased C availability (*Dambreville et al.*, 2006), whereas some authors observed an increase in N_2O emissions due to a higher denitrification activity (*Gutser et al.*, 2000; *Rochette et al.*, 2000) and other reports did not find any effect on N_2O release (*Meng et al.*, 2005).

Equally, whether the residual effect from prior organic fertilization and/or NI addition results in decreased yield-related GHG balance due to increased DM yields and N removal (*Alonso-Ayuso et al.*, 2016; *Schröder et al.*, 2007) or results in rising N losses which enhance GHG release is as yet unknown.

Although data collections in order to answer those questions and to reduce the uncertainty of the IPCC default value is a very time-intensive procedure, a determination of EFs considering different climatic conditions, soil types as well as the opportunity of fertilizer form-, application technique- and NI-specific adaption on regional base is indeed unavoidable to lay the foundation for further improvements of GHG inventories. Moreover, to ensure better comparability between future studies, the use of a sort of

standardized positive control for organic and mineral fertilization should be reconsidered.

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7 Curriculum Vitae

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Education

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04/2013-05/2015	**Master of Science** (Agricultural Sciences) University of Hohenheim, Stuttgart (Germany) Specialization: Crop production systems Thesis: „Einfluss von Rapsernterückständen auf umweltrelevante Nährstoffausträge" cumulative grade: very good (3.8 GPA)
10/2009-11/2012	**Bachelor of Science** (Agricultural Sciences) University of Hohenheim, Stuttgart (Germany) Specialization: Plant science Thesis: „Beruht der ys3 Mais-Genotyp mit gehemmter Phytosiderophor-(PS)-Abgabe auf einer Mutation des PS Exporter Gens?" cumulative grade: good (3.4 GPA)
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Other Education

07/2009-09/2009	Certified riding instructor (Trainer C), Orsingen-Nenzingen

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08/2013-09/2013	Internship in the field of marketing, Wacker Neuson Group in Munich (Germany)
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06/2016	Poster: Effect of application and nitrification inhibitors on trace gas fluxes from a maize field after cattle slurry amendment: 19th N workshop, Skara (Sweden)
09/2016	Poster: Effect of application and nitrification inhibitors on trace gas fluxes from a maize field after cattle slurry amendment: German Plant Nutrition 2016, International Conference, Stuttgart-Hohenheim (Germany)

Supervision of Bachelor Theses

03/2017	*Sebastian Franze*: Einfluss einer abfrierenden und einer winterharten Zwischenfrucht auf die N_2O-Emissionen im Winter
11/2017	*Meike Gassner*: Eignung von runden Gassammelhauben zur flächenrepräsentativen Erfassung von THG-Emissionen und Einfluss des Einsatzes eines Injektionsschars auf N_2O- und CH_4- Flüsse aus einer Parabraunerde
03/2018	*Philip Wichmann*: Einfluss verschiedener Umbruchtermine (UT) von Winterweizen auf N_2O-Emissionen in einem Feldversuch

Supervision of Master Theses

10/2017	*Sachin Aryal*: Effect of different nitrification inhibitors (NIs) and different history of NI application on N uptake by sunflower and N-mineralization in soils
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Elementary	Latin

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Hobbies	Traveling, riding, meeting friends

Stuttgart, 2 February 2019

Signature

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Eidesstattliche Versicherung gemäß § 8 Absatz 2 der Promotionsordnung der Universität Hohenheim zum Dr.sc.agr.

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Effects of nitrification inhibitors and application technique on trace gas fluxes from a maize field after cattle slurry fertilization

handelt es sich um meine eigenständig erbrachte Leistung.

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Ort und Datum Unterschrift

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Ich habe die Belehrung zur Eidesstattlichen Versicherung zur Kenntnis genommen.

Stuttgart, den 17. September 2018

Ort und Datum Unterschrift

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10 Appendix

10.1 Influence of NIs one year after application on crop N uptake in sunflower

10.1.1 Background and objective

Alonso-Ayuso et al. (2016) observed a significantly increased nitrogen (N) uptake of aboveground biomass from sunflower (*Helianthus annuus* L.) at flowering, providing that fertilization of the previous crop (*Zea mays* L.) in the year before, was conducted with mineral fertilizer in combination with the nitrification inhibitor (NI) 3,4-dimethylpyrazole phosphate (DMPP) instead of fertilizer application without NI. This study aimed to clarify whether NIs added to organic fertilization also have a N conservation effect as observed by *Alonso-Ayuso et al.* (2016). Thus, in an additional field experiment, the aim was to determine the residual effects from prior slurry injection with and without different NIs to silage maize. Therefore, sunflowers were cultivated from April till September 2016 without any fertilization on the study site on which treatment-specific fertilization of maize plants was conducted in May 2015 (for detailed description of fertilization see Chapter 3.3.1 and 4.3.3). To get more information about the N supply for sunflower over cropping season, harvesting was performed at flowering and physiological maturity. After each harvest, N contents and yield of aboveground biomass from sunflower heads and stems were quantified separately. A more detailed description of the experimental setup and procedure is given by *Aryal* (2017).

10.1.2 Results and Discussion

The initial mineral N levels in soil from plots with different history of organically fertilization (with or without NI addition) did not differ before sunflower cultivation (April 2016). Nevertheless, improved N removals in sunflower head and total crop at flowering and physiological maturity were observed, when prior fertilization was conducted with NIs, indicating a higher N supply (Table 10.1). At flowering, this putative increased N availability could be statistically secured for N removal of sunflower head and total crop when slurry injection was conducted with dicyandiamide (DCD) in the year before (Table 10.1). At physiological maturity, slurry injection with 1,2,4 Triazole & 3-Methylpyrazole (TZ & MP) and DMPP increased N removal in sunflower heads whereas the DMPP also recorded significant higher N removal in total crop compared to sole slurry injection one year earlier.

Table 10.1: Mean N uptake of sunflower head and total aboveground N at flowering and at physiological maturity as affected by fertilization in the preceding maize crop conducted one year before with or without differing nitrification inhibitors (*Student-Newman-Keuls test, $p<0.05$, $n=4$*).

Treatment*	N-uptake [kg N ha^{-1}]			
	At flowering		At physiological maturity	
	head	crop	head	crop
CON	28.8^{c}	59.8^{c}	47.3^{d}	68.1^{c}
INJ	38.6^{bc}	80.3^{bc}	77.4^{c}	109.8^{b}
INJ + DMPP	54.1^{ab}	112.6^{ab}	109.4^{a}	145.0^{a}
INJ + DMPSA	47.4^{ab}	98.5^{ab}	84.7^{bc}	116.6^{b}
INJ + Nitrayprin	52.4^{ab}	107.4^{ab}	78.8^{c}	119.9^{ab}
INJ + TZ & MP	46.4^{ab}	96.6^{ab}	101.7^{ab}	134.7^{ab}
INJ + DMPP & DMPSA	49.7^{ab}	103.3^{ab}	95.3^{abc}	128.7^{ab}
INJ + DCD	56.8^{a}	118.2^{a}	87.5^{bc}	119.3^{ab}

*CON= unfertilized control, INJ= cattle slurry injection. For further abbreviations see Table 4.1

In the similar field experiment, *Alonso-Ayuso et al.* (2016) used mineral fertilizer. They attributed the residual effect to the NI-induced N conservation in non-ready soil available forms of N which was fertilized to maize plants. They concluded that this N which was stabilized for at least one year was released to meet sunflower crop demand resulting in a mitigation of N losses.

10.1.3 References

Alonso-Ayuso, M., Gabriel, J. L., Quemada, M. (2016): Nitrogen use efficiency and residual effect of fertilizers with nitrification inhibitors. Eur. J. Agron. 80, 1–8.

Aryal, S. (2017): Effect of different nitrification inhibitors (NIs) and different history of NI application on N uptake by sunflower and N- mineralization in soils. Master thesis. University of Hohenheim, Germany.

10.2 High spatial resolution measurements with tripartite chambers compared to conventional circular chambers and the impact of slurry injection on N_2O and CH_4 release under wet spring conditions

10.2.1 Background and objectives

Similar to *Pfab* (2011) who used banded mineral fertilization, we conducted a study to evaluate the area-representative placement of circular chambers for greenhouse gas (GHG) measurements after slurry injection. Therefore, tripartite chambers were used (Figure 10.1). Each of the tripartite chambers was sampled separately to test how the GHG release from soil immediately affected by injection slot would differ from less or unaffected regions and if the circular chamber design used for our main study detects these putative differences in an area-representative way.

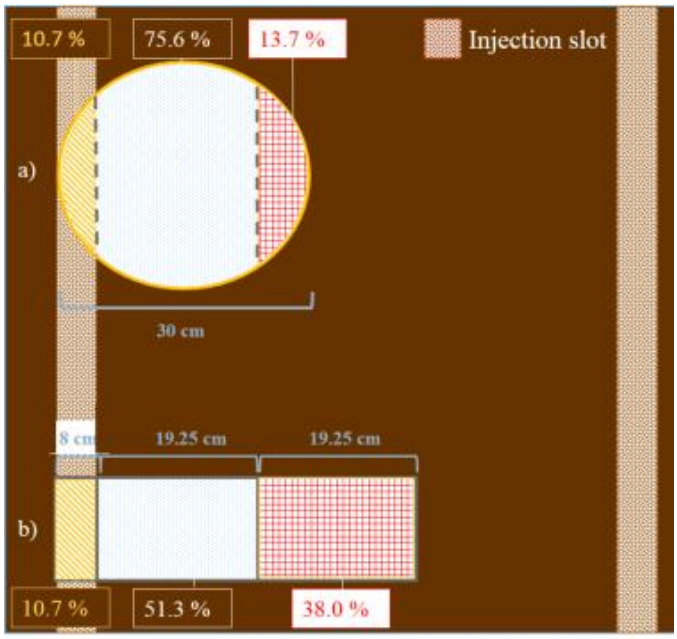

Figure 10.1: Size and position of circular (a) and tripartite (b) chambers on the injection slot.

Therefore, two similar cattle slurry injection treatments were conducted for GHG measurements, one with circular chamber design, the other one with tripartite chambers (fertilizer amount: 200 kg N ha^{-1}). Furthermore, a positive control on which the equivalent amount of water, adequate to the liquid volume of the slurry was inserted in the experimental design. In addition to GHG measurements from already existing site study during black fallow in 2017 (described in Chapter 3.3.1), in March 2017, this study was conducted immediately below the ongoing study. Experimental design was a randomized block design with four replicates. Distance between injection slots was 75 cm. For the calculation of the total cumulative N_2O loss, the area of each tripartite chamber was

taken into consideration (refer to Figure 10.1). A more detailed information of the experimental setup and procedure is given by *Gassner* (2017).

10.2.2 Results and Discussion

For slurry injection, over-estimation of GHG release from circular chamber design could be excluded and area-representative placement of circular base frames was confirmed through tripartite chambers (Figure 10.2). These results were also confirmed by *Pfab* (2011) for banded mineral fertilization.

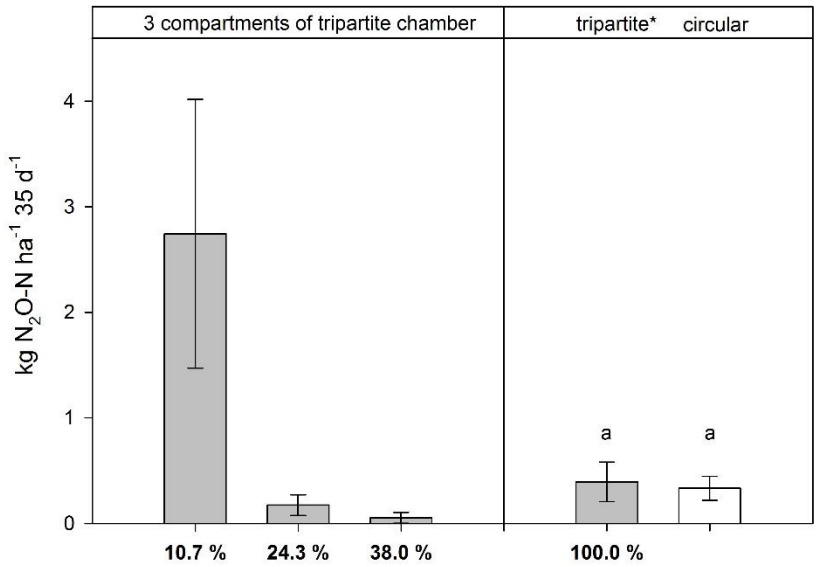

Figure 10.2: Cumulative N_2O-N emissions over 35 days from tripartite chambers from the compartments directly above slurry injection slot which covered 10.7 % of total plot area ("10.7 %"), from the compartment next to the slurry injection slot ("24.3 %") and from the compartment at a greater distance from the slurry injection slot ("38.0 %"); right: total cumulative N_2O-N emissions over 35 days from tripartite chambers related to total plot area and circular chambers (*n=4* for each; *for calculation the area of each tripartite chamber was taken into consideration). Statistically significant groups are indicated by different superscript letters (*Gassner*, 2017).

Beside the methodical evaluation of GHG measurements from banded organic fertilization when using circular chambers, increased methane (CH_4) emissions after slurry injection were observed for 35 days. *Gassner* (2017) attributed these high CH_4 emissions to methanogenesis. Net CH_4 uptake from positive control treatment with water application and the duration of CH_4 emissions confirmed this assumption of methanogenic fermentation processes through slurry application. Moreover, the delay of nitrification demonstrated anoxic soil conditions and led to postponed increase of nitrous oxide (N_2O) formation (Figure 10.3).

Finally, these observations illustrated that slurry injection on medium textured soils provokes N_2O and CH_4 emissions strongly, in particular under wet soil moisture contents in spring.

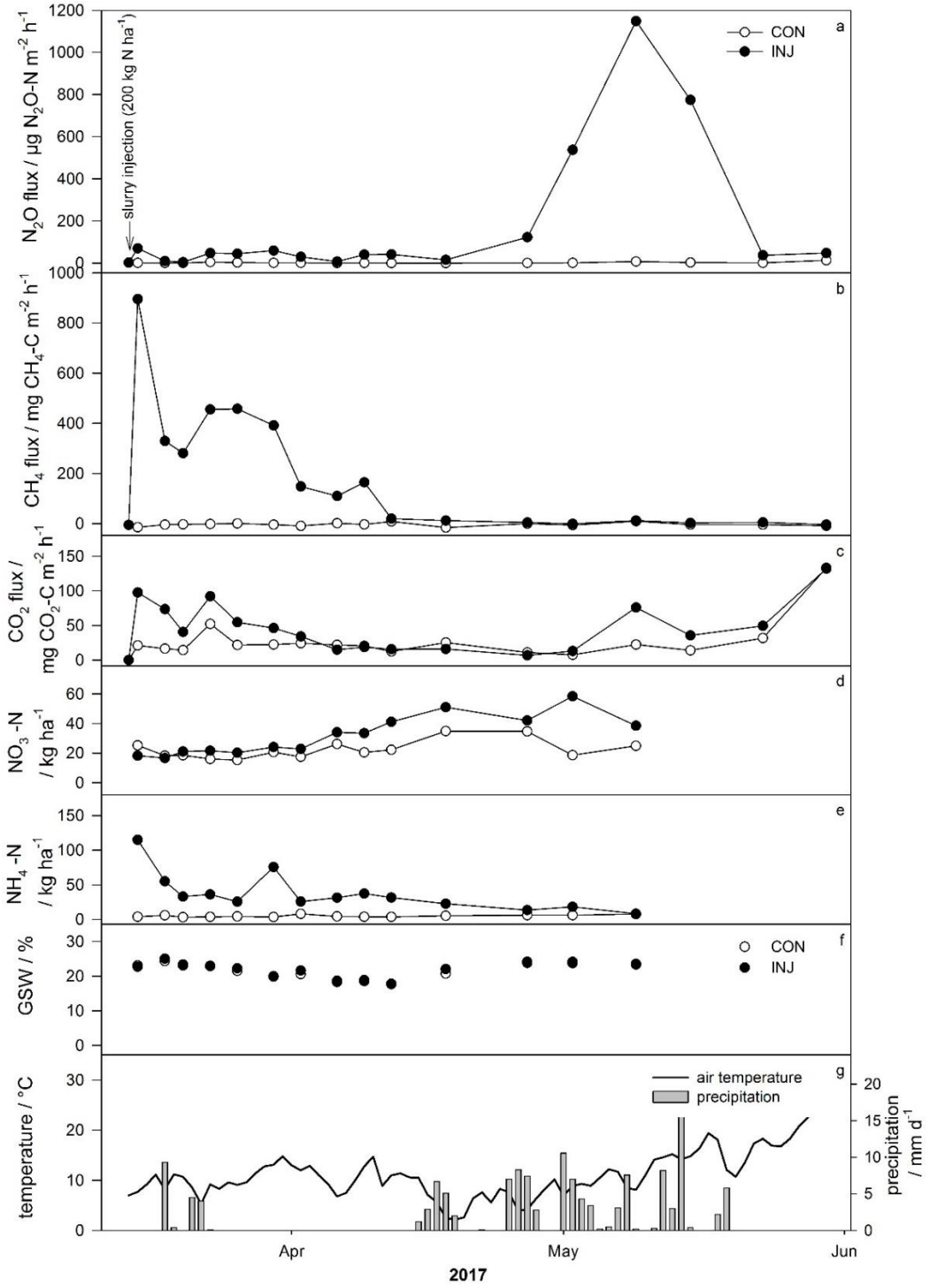

Figure 10.3: Mean N_2O flux rates (n=4) (a), mean CH_4 flux rates (n=4) (b), mean CO_2 flux rates (n=4) (c) from positive control with water application (CON) and slurry injection (INJ) as well as mean air temperature and daily precipitation (g) over the experimental period of 77 days (between 14 March and 6 June 2017). Both treatments were measured with circular chambers. Soil samples for determination of NO_3^--N contents (0-30 cm) (d), NH_4^+-N contents (0-30 cm) (e) and gravimetric soil water contents (GSW, 0-30 cm) (f) from CON and INJ treatment were taken between 14 March and 17 May 2017. Arrow indicates slurry or water application. Error bars are omitted for the purposes of clarity.

In accordance to German legislation on fertilization (*DüV*, 2017), the reduced time frame for organic fertilizer application might bear the risk of increased amounts applied under wet soil conditions in spring. Under these wet and relatively cool soil conditions, the use of nitrification inhibitors might be of particular importance.

10.2.3 References

DüV (German legislation on fertilization) (2017): Verordnung über die Anwendung von Düngemitteln, Bodenhilfsstoffen, Kultursubstraten und Pflanzenhilfsmitteln nach den Grundsätzen der guten fachlichen Praxis beim Düngen (Düngeverordnung – DüV). Bundesgesetzblatt Jahrgang 2017 Tl. I Nr. 32.

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